oligodeoxynucleotides into lesional skin reduced the parasite burdenapproximate to50-fold within the first 5 d of infection prior to full generation of a Th response. These results suggest that skin macrophages, constituting the principal reservoir of parasites in infected susceptible mice, produce Th1-promoting cytokines in response to **CpG**-containing immunostimulatory oligodeoxynucleotides. In addition, **CpG**-containing immunostimulatory oligodeoxynucleotides may also act locally on skin macrophages to facilitateLeishmania clearance by inducing nitric oxide production. (8 pages)

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=> s l13 and l14
L16         285 L13 AND L14

=> s l16 and asthma
L17         161 L16 AND ASTHMA

=> s l16 and allergy
L18         163 L16 AND ALLERGY

=> s l18 and phosphorothioate
L19          16 L18 AND PHOSPHOROTHIOATE

=> d bib ab 1-16

L19  ANSWER 1 OF 16  BIOSIS  COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN   1999:355473  BIOSIS
DN   PREV199900355473
TI   Oligodeoxynucleotides containing CpG motifs induce IL-12, IL-18
     and IFN-gamma production in cells from allergic individuals and inhibit
     IgE synthesis in vitro.
AU   Bohle, Barbara; Jahn-Schmid, Beatrice; Maurer, Dieter; Kraft, Dietrich;
     Ebner, Christof (1)
CS   (1) Department of General and Experimental Pathology, University of
     Vienna, Waehringer Guertel 18-20, A-1090, Vienna Austria
SO   European Journal of Immunology, (July, 1999) Vol. 29, No. 7, pp.
     2344-2353.
     ISSN: 0014-2980.
DT   Article
LA   English
SL   English
AB   The effects of phosphorothioate oligonucleotides containing
     CpG motifs (CpG-ODN) on cultured cells from allergic
     patients and non-atopic individuals were investigated. In peripheral blood
     mononuclear cells (PBMC) CpG-ODN led to a significant increase
     of IFN-gamma. By intracellular cytokine staining, IFN-gamma production
     could be attributed to NK cells and inhibition experiments indicated an
     IL-12-dependent mechanism. Moreover, CpG-ODN increased mRNA
     expression of IL-12 and IL-18 in PBMC. In this respect, no significant
     difference between allergic and non-atopic individuals was observed.
     Monocyte-derived dendritic cells were identified as one IL-12- and
     IL-18-producing source. In addition, stimulation of PBMC derived from
     atopic patients with CpG-ODN led to a considerable increase of
     polyclonal IgG and IgM synthesis. In contrast, the production of total IgE
     was suppressed. CpG-ODN induced a significant rise of IgG and
     IgM specific for allergens to which the patients were sensitized, whereas
     allergen-specific IgE levels remained unchanged. Our data suggest that
     CpG-ODN display a strong influence on the ongoing immune response
     and might represent potential adjuvants for specific immunotherapy of type
     I allergy.

L19  ANSWER 2 OF 16  EMBASE  COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
AN   2001331889  EMBASE
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TI Immunostimulatory DNA inhibits IL-4-dependent IgE synthesis by human B cells.
AU Horner A.A.; Widhopf G.F.; Burger J.A.; Takabayashi K.; Cinman N.; Ronaghy A.; Spiegelberg H.L.; Raz E.
CS Dr. A.A. Horner, University of California, 9500 Gilman Dr., San Diego, CA 92093-0663, United States
SO Journal of Allergy and Clinical Immunology, (2001) 108/3 (417-423).
Refs: 36
ISSN: 0091-6749 CODEN: JACIBY
CY United States
DT Journal; Article
FS 026 Immunology, Serology and Transplantation
LA English
SL English
AB Background: Immunostimulatory sequence oligodeoxynucleotide (ISS-ODN) is a potent antiallergic immunomodulating agent in mice. However, few studies have addressed its antiallergic potential in human subjects. Objective: We sought to determine whether a **phosphorothioate** ISS-ODN could inhibit IL-4-dependent IgE synthesis by human B cells. Methods: Initially, nonatopic- and atopic-donor PBMCs were incubated with ISS-ODN or mutated oligodeoxynucleotide, and cytokine production and B-cell expression of IFN-.gamma. receptor and IL-4 receptor were measured by using ELISA and flow cytometry, respectively. In subsequent studies atopic-donor PBMCs were incubated with IL-4 alone or with ISS-ODN or mutated oligodeoxynucleotide. After 14 days, IgE production and IgM, IgG, and IgA production were determined by using ELISA. In select IgE studies cytokines were neutralized with mAbs. Results: ISS-ODN induced IL-12, IFN-.alpha. IFN-.gamma., IL-10, and IL-6 production from both nonatopic- and atopic-donor PBMCs. ISS-ODN also increased IFN-.gamma. receptor and inhibited IL-4 receptor expression on B cells from both donor populations. Furthermore, ISS-ODN inhibited IL-4-dependent IgE production by atopic-donor PBMCs. Neutralization of IL-12, IFN-.alpha., IFN-.gamma., and IL-10, but not IL-6, attenuated the inhibitory activity of ISS-ODN on IgE production. In contrast to its inhibition of IgE synthesis, ISS-ODN stimulated the production of IgM, IgG, and IgA. Conclusion: These in vitro studies demonstrate that **phosphorothioate** ISS-ODN elicits an innate immune response by PBMCs, which inhibits IL-4-dependent IgE synthesis. In addition, these results provide further support for consideration of ISS-ODN therapy for the treatment of allergic disease in clinical practice.

L19 ANSWER 3 OF 16 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
AN 1999408447 EMBASE
TI **Phosphorothioate** oligodeoxynucleotides promote the in vitro development of human allergen-specific CD4+ T cells into Th1 effectors.
AU Parronchi P.; Brugnolo F.; Annunziato F.; Manuelli C.; Sampognaro S.; Mavilia C.; Romagnani S.; Maggi E.
CS Dr. S. Romagnani, Dipartimento di Medicina Interna, Sezione di Immunoallergologia, Policlinico di Careggi, Viale Morgagni 85, 50134 Florence, Italy. s.romagnani@mednuc2.dfc.unifi.it
SO Journal of Immunology, (1 Dec 1999) 163/11 (5946-5953).
Refs: 41
ISSN: 0022-1767 CODEN: JOIMA3
CY United States
DT Journal; Article
FS 026 Immunology, Serology and Transplantation
037 Drug Literature Index
LA English
SL English
AB DNA vaccination is an effective approach in inducing the switch of murine immune responses from a Th2 to a Th1 profile of cytokine production that has been related to the activity of unmethylated **CpG** motifs present in bacterial, but not mammalian, DNA. We report here that some synthetic **phosphorothioate**, but not phosphodiester,

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=> s l9 and (refractory or hyporesponsive or hypo-responsive or non-responsive)
L11       1292 L9 AND (REFRACTORY OR HYPORESPONSIVE OR HYPO-RESPONSIVE OR
               NON-RESPONSIVE)

=> dup rem l11
PROCESSING IS APPROXIMATELY  85% COMPLETE FOR L11
PROCESSING COMPLETED FOR L11
L12         785 DUP REM L11 (507 DUPLICATES REMOVED)

=> s l9 and l10
L13         523 L9 AND L10

=> dup rem l13
PROCESSING COMPLETED FOR L13
L14         285 DUP REM L13 (238 DUPLICATES REMOVED)

=> s l12 and l14
L15           1 L12 AND L14

=> d bib ab

L15   ANSWER 1 OF 1  BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
AN    2002-16855  BIOTECHDS
TI    Skin-derived macrophages from Leishmania major-susceptible mice exhibit
      interleukin-12-and interferon-gamma-independent nitric oxide production
      and parasite killing after treatment with immunostimulatory DNA;
         useful for infectious disease, tumor, autoimmune disease,
         allergy gene therapy and nucleic acid vaccine
AU    VON STEBUT E; BELKAID Y; NGUYEN B; WILSON M; SACKS DL; UDEY MC
CS    Univ Mainz; NIAID; NCI
LO    von Stebut E, Univ Mainz, Dept Dermatol, Langenbeckstr 1, D-55131 Mainz,
      Germany
SO    JOURNAL OF INVESTIGATIVE DERMATOLOGY; (2002) 119, 3, 621-628    ISSN:
      0022-202X
DT    Journal
LA    English
AB    AUTHOR ABSTRACT - Co-administration of CpG-containing
      immunostimulatory oligodeoxynucleotidesand parasite antigen protects
      susceptible BALB/c mice from otherwise progressive infection with
      Leishmania major. Although the protective effect of CpG
      -containing immunostimulatory oligodeoxynucleotides is clearly
      dependenton endogenous interleukin-12 and interferon-gamma production,
      the source ofthese Th1-promoting cytokines in infected mice is unknown.
      In contrast tomacrophages from Leishmania-resistant C57BL/6 mice,
      macrophages from susceptible BALB/c mice are hyporesponsive to
      stimulation with lipopolysaccharideand interferon-gamma. While studying
      interactions of various antigen-presenting cells with Leishmania, we
      found that BALB/c inflammatory skin macrophages, whether
      Leishmania-infected or uninfected, produced large amounts
      ofinterleukin-12 when treated with CpG-containing
      immunostimulatory oligodeoxynucleotides. Like lipopolysaccharide,
      CpG-containing immunostimulatory oligodeoxynucleotides induced
      production of interferon-gamma and release of nitric oxide by skin
      macrophages. Studies using skin macrophages from interleukin-12- and
      interferon-gamma-deficient BALB/c mice demonstrated that nitric oxide
      release was not dependent on interleukin-12 and interferon-gamma
      production. Approximately 44% and 27% of intracellular Leishmania major
      amastigotes were killed by infected skin macrophages within 72 h upon
      stimulation with CpG-containing immunostimulatory
      oligodeoxynucleotides and lipopolysaccharide, respectively. Parasite
      killing by macrophages was independent ofendogenous interferon-gamma
      production, but was strongly enhanced by exogenous interferon-gamma.
      Parasite elimination was dependent on the inductionof nitric oxide,
      however. In vivo, injection of CpG-containing immunostimulatory
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United States Patent Application *20010044416*
Kind Code **A1**
McCluskie, Michael J. ; et al. **November 22, 2001**

Immunostimulatory nucleic acids for inducing a Th2 immune response

Abstract

The invention relates to methods and products for inducing an immune response using immunostimulatory nucleic acids. In particular the immunostimulatory nucleic acids preferentially induce a Th2 immune response. The invention is useful for treating and preventing disorders associated with a Th1 immune response or for creating a Th2 environment for treating disorders that are sensitive to Th2 immune responses.

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Serial No.: **768012**
Series Code: **09**
Filed: **January 22, 2001**

U.S. Current Class: **514/44**; 514/110; 514/179; 514/9
U.S. Class at Publication: **514/44**; 514/9; 514/179; 514/110
Intern'l Class: A61K 048/00; A61K 031/675; A61K 038/13; A61K 031/573

Claims

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We claim:

1. A method for inducing an antigen specific response comprising: administering to a subject an antigen and a Th2-immunostimulatory nucleic acid in an amount effective to produce an antigen specific immune response when the Th2-immunostimulatory nucleic acid is administered mucosally or dermally.

2. The method of claim 1, wherein the subject is administered the antigen after the Th2-immunostimulatory nucleic acid.

3. The method of claim 1, wherein the subject is administered the antigen before the Th2-immunostimulatory nucleic acid.

4. The method of claim 1, wherein the subject is administered the antigen and the Th2-immunostimulatory nucleic acid simultaneously.

5. The method of claim 1, wherein the Th2-immunostimulatory nucleic acid is delivered to the mouth, skin or eye.

6. The method of claim 1, further comprising administering a therapeutic agent to the subject.

7. The method of claim 6, wherein the therapeutic agent is a Th1 adjuvant.

8. The method of claim 7, wherein the Th1 adjuvant is selected from the group consisting of CpG nucleic acids, MF59, SAF, MPL, and QS21.

9. The method of claim 7, wherein the Th1 adjuvant is administered following the administration of the Th2-immunostimulatory nucleic acid.

10. The method of claim 6, wherein the therapeutic agent is a Th2 adjuvant.

11. The method of claim 10, wherein the Th2 adjuvant is selected from the group consisting of adjuvants that create a depot effect, adjuvants that stimulate the immune system, and adjuvants that create a depot effect and stimulate the immune system and mucosal adjuvants.

12. The method of claim 11, wherein the adjuvant that creates a depot effect is selected from the group consisting of alum; emulsion-based formulations including mineral oil, non-mineral oil, water-in-oil or oil-in-water-in oil emulsion, oil-in-water emulsions such as Seppic ISA series of Montanide adjuvants; and PROVAX.

13. The method of claim 11, wherein the adjuvant that stimulates the immune system is selected from the group consisting of saponins purified from the bark of the Q. saponaria tree; poly[di (carboxylatophenoxy)phosph- azene; derivatives of lipopolysaccharides, muramyl dipeptide and threonyl-muramyl dipeptide; OM-174; and Leishmania elongation factor.

14. The method of claim 11, wherein the adjuvant that creates a depot effect and stimulates the immune system is selected from the group consisting of ISCOMs; SB-AS2; SB-AS4; non-ionic block copolymers that form micelles such as CRL 1005; and Syntex Adjuvant Formulation.

15. The method of claim 11, wherein the mucosal adjuvant is selected from the group consisting of CpG

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nucleic acids, Bacterial toxins, Cholera toxin, CT derivatives, CT B subunit; CTD53; CTK97; CTK104; CTD53/K63; CTH54; CTN107; CTE114; CTE112K; CTS61F; CTS106; and CTK63, Zonula occludens toxin, zot, Escherichia coli heat-labile enterotoxin, Labile Toxin, LT derivatives, LT B subunit; LT7K; LT61F; LT112K; LT118E; LT146E; LT192G; LTK63; and LTR72, Pertussis toxin, PT-9K/129G; Toxin derivatives; Lipid A derivatives, MDP derivatives; Bacterial outer membrane proteins, outer surface protein A (OspA) lipoprotein of Borrelia burgdorferi, outer membrane protein of Neisseria meningitidis; Oil-in-water emulsions, Aluminum salts; and Saponins, ISCOMs, the Seppic ISA series of Montanide adjuvants, Montanide ISA 720; PROVAX; Syntext Adjuvant Formulation; poly[di (carboxylatophenoxy) phosphazene and Leishmania elongation factor.

16. The method of claim 6, wherein the therapeutic agent is a cytokine.

17. The method of claim 1, wherein the Th2-immunostimulatory nucleic acid is formulated in a form selected from the group consisting of a liquid solution, a powder, a microparticle, and a bioadhesive polymer.

18. The method of claim 1, wherein the Th2-immunostimulatory nucleic acid is administered by a route selected from the group consisting of oral, intranasal, vaginal, rectal, intra-ocular, and by inhalation.

19. The method of claim 1, wherein the Th2-immunostimulatory nucleic acid is administered by a route selected from the group consisting of intradermal, intraepidermal and transdermal.

20. The method of claim 1, wherein the antigen specific immune response is a systemic immune response.

21. The method of claim 1, wherein the antigen specific immune response is a mucosal immune response.

22. The method of claim 1, wherein the Th2-immunostimulatory nucleic acid is administered using a delivery system selected from the group consisting of a needleless delivery system, a scarification delivery system, and a tyne delivery system.

23. The method of claim 1, wherein the antigen is administered using a delivery system selected from the group consisting of a needleless delivery system, a scarification delivery system, and a tyne delivery system.

24. The method of claim 6, wherein the therapeutic agent is selected from the group consisting of an anti-viral agent, an anti-bacterial agent, an anti-parasitic agent, an anti-fungal agent, and cancer medicament.

25. The method of claim 1, wherein the antigen is selected from the group of antigens consisting of viral antigens, fungal antigens, bacterial antigens, parasitic antigens, and cancer antigens.

26. The method of claim 1, wherein the subject has not been exposed to an Th1 immunostimulatory nucleic acid prior to administration of the Th2 immunostimulatory nucleic acid.

27. The method of claim 1, wherein the subject is not experiencing a Th1 mediated disorder at the time of administration.

28. The method of claim 1, wherein the antigen is not conjugated to the Th2 immunostimulatory nucleic acid.

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29. The method of claim 1, wherein the antigen is not a self antigen.

30. The method of claim 1, wherein the antigen is not an extracellular antigen.

31. A method for inducing an antigen specific response comprising: administering to a subject an antigen and a Th2-immunostimulatory nucleic acid in an amount effective to produce an antigen specific immune response when the Th2-immunostimulatory nucleic acid is administered parenterally.

32. The method of claim 31, wherein the subject is administered the antigen after the Th2-immunostimulatory nucleic acid.

33. The method of claim 31, wherein the subject is administered the antigen before the Th2-immunostimulatory nucleic acid.

34. The method of claim 31, wherein the subject is administered the antigen and the Th2-immunostimulatory nucleic acid simultaneously.

35. The method of claim 31, wherein the Th2-immunostimulatory nucleic acid is delivered intravenously, intraperitoneally, intramuscularly, subcutaneously, or by infusion.

36. The method of claim 31, further comprising administering a therapeutic agent to the subject.

37. The method of claim 36, wherein the therapeutic agent is a Th1 adjuvant.

38. The method of claim 37, wherein the Th1 adjuvant is selected from the group consisting of CpG nucleic acids, MF59, SAF, MPL, and QS21.

39. The method of claim 36, wherein the therapeutic agent is a Th2 adjuvant.

40. The method of claim 39, wherein the Th2 adjuvant is selected from the group consisting of adjuvants that creates a depot effect, adjuvants that stimulate the immune system, adjuvants that create a depot effect and stimulate the immune system and mucosal adjuvants.

41. The method of claim 40, wherein the adjuvant that creates a depot effect is selected from the group consisting of alum; emulsion-based formulations including mineral oil, non-mineral oil, water-in-oil or oil-in-water-in oil emulsion, oil-in-water emulsions such as Seppic ISA series of Montanide adjuvants; and PROVAX.

42. The method of claim 40, wherein the adjuvant that stimulates the immune system is selected from the group consisting of saponins purified from the bark of the Q. saponaria tree; poly[di (carboxylatophenoxy)phosph- azene; derivatives of lipopolysaccharides, muramyl dipeptide and threonyl-muramyl dipeptide; OM-174; and Leishmania elongation factor.

43. The method of claim 40, wherein the adjuvant that creates a depot effect and stimulates the immune system is selected from the group consisting of ISCOMs; SB-AS2; SB-AS4; non-ionic block copolymers that form micelles such as CRL 1005; and Syntex Adjuvant Formulation.

44. The method of claim 40, wherein the mucosal adjuvant is selected from the group consisting of CpG nucleic acids, Bacterial toxins, Cholera toxin, CT derivatives, CT B subunit; CTD53; CTK97; CTK104; CTD53/K63; CTH54; CTN107; CTE114; CTE112K; CTS61F; CTS106; and CTK63, Zonula occludens

toxin, zot, Escherichia coli heat-labile enterotoxin, Labile Toxin, LT derivatives, LT B subunit; LT7K; LT61F; LT112K; LT118E; LT146E; LT192G; LTK63; and LTR72, Pertussis toxin, PT-9K/129G; Toxin derivatives; Lipid A derivatives, MDP derivatives; Bacterial outer membrane proteins, outer surface protein A (OspA) lipoprotein of Borrelia burgdorferi, outer membrane protein of Neisseria meningitidis; Oil-in-water emulsions, Aluminum salts; and Saponins, ISCOMs, the Seppic ISA series of Montanide adjuvants, Montanide ISA 720; PROVAX; Syntext Adjuvant Formulation; poly[di (carboxylatophenoxy) phosphazene and Leishmania elongation factor.

45. The method of claim 36, wherein the therapeutic agent is a cytokine.

46. The method of claim 31, wherein the Th2-immunostimulatory nucleic acid is formulated in a form selected from the group consisting of a liquid solution, a powder, a microparticle, and a bioadhesive polymer.

47. The method of claim 31, wherein the antigen is a non-extracellular antigen.

48. The method of claim 31, wherein the antigen specific immune response is a systemic immune response.

49. The method of claim 31, wherein the antigen is administered using a delivery system selected from the group consisting of a needleless delivery system, a scarification delivery system, and a tyne delivery system.

50. The method of claim 36, wherein the therapeutic agent is selected from the group consisting of an anti-viral agent, an anti-bacterial agent, an anti-parasitic agent, an anti-fungal agent, and cancer medicament.

51. The method of claim 31, wherein the antigen is selected from the group of antigens consisting of viral antigens, fungal antigens, yeast antigens, parasitic antigens, and tumor (i.e., cancer) antigens.

52. The method of claim 31, wherein the subject has not been exposed to an Th1 immunostimulatory nucleic acid prior to administration of the Th2 immunostimulatory nucleic acid.

53. The method of claim 31, wherein the antigen is not conjugated to the Th2 immunostimulatory nucleic acid.

54. The method of claim 31, wherein the antigen is not a self antigen.

55. A method for treating a non-autoimmune Th1-mediated disease, comprising: administering to a subject a Th2 immunostimulatory nucleic acid in an amount effective to produce a Th2 immune response when administered mucosally or dermally.

56. The method of claim 55, wherein an antigen is not administered to the subject.

57. The method of claim 55, wherein the subject has not been exposed to a Th1 immunostimulatory nucleic acid.

58. The method of claim 55, wherein the non-autoimmune Th1-mediated disease is not mediated by a Th1 immunostimulatory nucleic acid.

59. The method of claim 56, wherein the disorder is selected from the group consisting of psoriasis, Th1

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inflammatory disorders, solid organ allograft rejection, symptoms associated with Hepatitis B infection, insulin-dependent diabetes mellitus, multiple sclerosis, "Silent thyroiditis", and unexplained recurrent abortion.

60. The method of claim 55, wherein the method is a method for inducing a local Th2 environment in the subject.

61. The method of claim 60, wherein the local Th2 environment is in the skin and wherein the subject has a Th1 mediated skin disorder.

62. The method of claim 60, wherein the local Th2 environment is in the eye and the subject has a viral infection.

63. The method of claim 62, wherein the viral infection is HSV-1.

64. The method of claim 55, wherein the Th2-immunostimulatory nucleic acid is administered locally.

65. The method of claim 64, wherein the Th2-immunostimulatory nucleic acid is administered to a tissue selected from the group consisting of skin and eye.

66. The method of claim 55, further comprising administering a therapeutic agent to the subject.

67. The method of claim 66, wherein the therapeutic agent is a Th1 adjuvant.

68. The method of claim 67, wherein the Th1 adjuvant is selected from the group consisting of CpG nucleic acids, MF59, SAF, MPL, and QS21.

69. The method of claim 66, wherein the therapeutic agent is a Th2 adjuvant.

70. The method of claim 69, wherein the Th2 adjuvant is selected from the group consisting of adjuvants that creates a depot effect, adjuvants that stimulate the immune system, adjuvants that create a depot effect and stimulate the immune system and mucosal adjuvants.

71. The method of claim 70, wherein the adjuvant that creates a depot effect is selected from the group consisting of alum; emulsion-based formulations including mineral oil, non-mineral oil, water-in-oil or oil-in-water-in oil emulsion, oil-in-water emulsions such as Seppic ISA series of Montanide adjuvants; and PROVAX.

72. The method of claim 70, wherein the adjuvant that stimulates the immune system is selected from the group consisting of saponins purified from the bark of the Q. saponaria tree; poly[di (carboxylatophenoxy)phosph- azene; derivatives of lipopolysaccharides, muramyl dipeptide and threonyl-muramyl dipeptide; OM-174; and Leishmania elongation factor.

73. The method of claim 70, wherein the adjuvant that creates a depot effect and stimulates the immune system is selected from the group consisting of ISCOMs; SB-AS2; SB-AS4; non-ionic block copolymers that form micelles such as CRL 1005; and Syntex Adjuvant Formulation.

74. The method of claim 70, wherein the mucosal adjuvant is selected from the group consisting of CpG nucleic acids, Bacterial toxins, Cholera toxin, CT derivatives, CT B subunit; CTD53; CTK97; CTK104; CTD53/K63; CTH54; CTN107; CTE114; CTE112K; CTS61F; CTS106; and CTK63, Zonula occludens toxin, zot, Escherichia coli heat-labile enterotoxin, Labile Toxin, LT derivatives, LT B subunit; LT7K;

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LT61F; LT112K; LT118E; LT146E; LT192G; LTK63; and LTR72, Pertussis toxin, PT-9K/129G; Toxin derivatives; Lipid A derivatives, MDP derivatives; Bacterial outer membrane proteins, outer surface protein A (OspA) lipoprotein of Borrelia burgdorferi, outer membrane protein of Neisseria meningitidis; Oil-in-water emulsions, Aluminum salts; and Saponins, ISCOMs, the Seppic ISA series of Montanide adjuvants, Montanide ISA 720; PROVAX; Syntext Adjuvant Formulation; poly[di (carboxylatophenoxy) phosphazene and Leishmania elongation factor.

75. The method of claim 66, wherein the therapeutic agent is a cytokine.

76. The method of claim 66, wherein the therapeutic agent is a drug for treating Th1 mediated disorders.

77. The method of claim 76, wherein the drug for treating Th1 mediated disorders is selected from the group consisting of anti-psoriasis creams, eye drops, nose drops, Sulfasalazine, glucocorticoids, propylthiouracil, methimazole, .sup.131, insulin, IFN-.beta.1a, IFN-.beta.1b, copolymer 1 (i.e., MS), glucocorticoids (i.e., MS), ACTH, avonex, azathioprine, cyclophosphamide, UV-B, PUVA, methotrexate, calcipitriol, cyclophosphamide, OKT3, FK-506, cyclosporin A, azathioprine, and mycophenolate mofetil.

78. A method for treating an autoimmune disease, comprising: administering to a subject a Th2-immunostimulatory nucleic acid in an amount effective to produce a Th2 immune response when administered mucosally or dermally, wherein the subject has not been exposed to a Th1 immunostimulatory nucleic acid.

79. The method of claim 78, wherein the autoimmune disease is selected from the group consisting of rheumatoid arthritis, Crohn's disease, systemic lupus erythematosus (SLE), autoimmune encephalomyelitis, myasthenia gravis, Hashimoto's thyroiditis, Goodpasture's syndrome, pemphigus, Grave's disease, autoimmune hemolytic anemia, autoimmune thrombocytopenic purpura, scleroderma with anti-collagen antibodies, mixed connective tissue disease, polymyositis, pernicious anemia, idiopathic Addison's disease, autoimmune-associated infertility, glomerulonephritis, bullous pemphigoid, Sjogren's syndrome, insulin resistance, and autoimmune diabetes mellitus.

80. The method of claim 78, further comprising administering to the subject a self antigen, to produce an immune hyporesponsive state.

81. The method of claim 80, wherein the self antigen is not conjugated to the Th2 immunostimulatory nucleic acid.

82. The method of claim 78, wherein the method is a method for inducing a local Th2 environment in the subject.

83. The method of claim 82, wherein the local Th2 environment is in the skin.

84. The method of claim 82, wherein the local Th2 environment is in the eye.

85. The method of claim 78, wherein the Th2-immunostimulatory nucleic acid is administered mucosally.

86. The method of claim 78, wherein the Th2-immunostimulatory nucleic acid is administered locally.

87. The method of claim 86, wherein the Th2-immunostimulatory nucleic acid is administered to a

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tissue selected from the group consisting of skin and eye.

88. The method of claim 78, further comprising administering a therapeutic agent to the subject.

89. The method of claim 88, wherein the therapeutic agent is a Th1 adjuvant.

90. The method of claim 89, wherein the Th1 adjuvant is selected from the group consisting of CpG nucleic acids, MF59, SAF, MPL, and QS21.

91. The method of claim 88, wherein the therapeutic agent is a Th2 adjuvant.

92. The method of claim 91, wherein the Th2 adjuvant is selected from the group consisting of adjuvants that creates a depot effect, adjuvants that stimulate the immune system, adjuvants that create a depot effect and stimulate the immune system and mucosal adjuvants.

93. The method of claim 92, wherein the adjuvant that creates a depot effect is selected from the group consisting of alum; emulsion-based formulations including mineral oil, non-mineral oil, water-in-oil or oil-in-water-in oil emulsion, oil-in-water emulsions such as Seppic ISA series of Montanide adjuvants; and PROVAX.

94. The method of claim 92, wherein the adjuvant that stimulates the immune system is selected from the group consisting of saponins purified from the bark of the Q. saponaria tree; poly[di (carboxylatophenoxy)phosph- azene; derivatives of lipopolysaccharides, muramyl dipeptide and threonyl-muramyl dipeptide; OM-174; and Leishmania elongation factor.

95. The method of claim 92, wherein the adjuvant that creates a depot effect and stimulates the immune system is selected from the group consisting of ISCOMs; SB-AS2; SB-AS4; non-ionic block copolymers that form micelles such as CRL 1005; and Syntex Adjuvant Formulation.

96. The method of claim 92, wherein the mucosal adjuvant is selected from the group consisting of CpG nucleic acids, Bacterial toxins, Cholera toxin, CT derivatives, CT B subunit; CTD53; CTK97; CTK104; CTD53/K63; CTH54; CTN107; CTE114; CTE112K; CTS61F; CTS106; and CTK63, Zonula occludens toxin, zot, Escherichia coli heat-labile enterotoxin, Labile Toxin, LT derivatives, LT B subunit; LT7K; LT6 IF; LT112K; LT118E; LT146E; LT192G; LTK63; and LTR72, Pertussis toxin, PT-9K/129G; Toxin derivatives; Lipid A derivatives, MDP derivatives; Bacterial outer membrane proteins, outer surface protein A (OspA) lipoprotein of Borrelia burgdorferi, outer membrane protein of Neisseria meningitidis; Oil-in-water emulsions, Aluminum salts; and Saponins, ISCOMs, the Seppic ISA series of Montanide adjuvants, Montanide ISA 720; PROVAX; Syntext Adjuvant Formulation; poly[di (carboxylatophenoxy) phosphazene and Leishmania elongation factor.

97. The method of claim 88, wherein the therapeutic agent is a cytokine.

98. The method of claim 88, wherein the therapeutic agent is a drug for treating autoimmune disease.

99. The method of claim 98, wherein the drug for treating Th1 mediated disorders is selected from the group consisting of anti-psoriasis creams, eye drops, nose drops, Sulfasalazine, glucocorticoids, propylthiouracil, methimazole, .sup.131, insulin, IFN-.beta.1a, IFN-.beta.1b, copolymer 1 (i.e., MS), glucocorticoids (i.e., MS), ACTH, avonex, azathioprine, cyclophosphamide, UV-B, PUVA, methotrexate, calcipitriol, cyclophosphamide, OKT3, FK-506, cyclosporin A, azathioprine, and mycophenolate mofetil.

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100. A pharmaceutical composition, comprising: an effective amount of a Th2 immunostimulatory nucleic acid for stimulating a Th2 immune response when administered mucosally or dermally, an antigen, and a pharmaceutically acceptable carrier.

101. The pharmaceutical composition of claim 100, wherein the antigen is not conjugated to the Th2 immunostimulatory nucleic acid.

102. The pharmaceutical composition of claim 100, wherein the Th2 immune response is a mucosal immune response.

103. The pharmaceutical composition of claim 100, wherein the Th2 immune response is a systemic immune response.

104. The pharmaceutical composition of claim 100, wherein the antigen is not an self antigen.

105. The pharmaceutical composition of claim 100, wherein the Th2-immunostimulatory nucleic acid is formulated in a delivery vehicle selected from the group consisting of bioadhesive polymers, cochleates, dendrimers, enteric-coated capsules, emulsomes, ISCOMs, liposomes, cationic lipids, microspheres, nanospheres, polymer rings, proteosomes, and virosomes.

106. The pharmaceutical composition of claim 100, further comprising a therapeutic agent.

107. The pharmaceutical composition of claim 106, wherein the therapeutic agent is a Th1 adjuvant.

108. The pharmaceutical composition of claim 106, wherein the therapeutic agent is a Th2 adjuvant.

109. The pharmaceutical composition of claim 106, wherein the therapeutic agent is a cytokine.

110. The pharmaceutical composition of claim 106, wherein the therapeutic agent is a drug for treating Th1 mediated disorders.

111. The pharmaceutical composition of claim 105, wherein the Th2-immunostimulatory nucleic acid and antigen are present in different delivery vehicles.

112. A pharmaceutical composition, comprising: an effective amount of a Th2 immunostimulatory nucleic acid for stimulating a Th2 immune response when administered mucosally or dermally, and an adjuvant, in a pharmaceutically acceptable carrier.

113. The pharmaceutical composition of claim 112, wherein the Th2 immune response is a mucosal immune response.

114. The pharmaceutical composition of claim 112, wherein the Th2 immune response is a systemic immune response.

115. The pharmaceutical composition of claim 112, wherein the adjuvant is a Th1 adjuvant.

116. The pharmaceutical composition of claim 112, wherein the Th1 adjuvant is selected from the group consisting of CpG nucleic acids, MF59, SAF, MPL, and QS21.

117. The pharmaceutical composition of claim 112, wherein the adjuvant is a Th2 adjuvant.

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118. The pharmaceutical composition of claim 117, wherein the Th2 adjuvant is selected from the group consisting of adjuvants that creates a depot effect, adjuvants that stimulate the immune system, adjuvants that create a depot effect and stimulate the immune system and mucosal adjuvants.

119. The pharmaceutical composition of claim 118, wherein the adjuvant that creates a depot effect is selected from the group consisting of alum; emulsion-based formulations including mineral oil, non-mineral oil, water-in-oil or oil-in-water-in oil emulsion, oil-in-water emulsions such as Seppic ISA series of Montanide adjuvants; and PROVAX.

120. The pharmaceutical composition of claim 118, wherein the adjuvant that stimulates the immune system is selected from the group consisting of saponins purified from the bark of the Q. saponaria tree; poly[di(carboxylatophenoxy)phosphazene; derivatives of lipopolysaccharides, muramyl dipeptide and threonyl-muramyl dipeptide; OM-174; and Leishmania elongation factor.

121. The pharmaceutical composition of claim 118, wherein the adjuvant that creates a depot effect and stimulates the immune system is selected from the group consisting of ISCOMs; SB-AS2; SB-AS4; non-ionic block copolymers that form micelles such as CRL 1005; and Syntex Adjuvant Formulation.

122. The pharmaceutical composition of claim 118, wherein the mucosal adjuvant is selected from the group consisting of CpG nucleic acids, Bacterial toxins, Cholera toxin, CT derivatives, CT B subunit; CTD53; CTK97; CTK104; CTD53/K63; CTH54; CTN107; CTE114; CTE112K; CTS61F; CTS106; and CTK63, Zonula occludens toxin, zot, Escherichia coli heat-labile enterotoxin, Labile Toxin, LT derivatives, LT B subunit; LT7K; LT61F; LT112K; LT118E; LT146E; LT192G; LTK63; and LTR72, Pertussis toxin, PT-9K/129G; Toxin derivatives; Lipid A derivatives, MDP derivatives; Bacterial outer membrane proteins, outer surface protein A (OspA) lipoprotein of Borrelia burgdorferi, outer membrane protein of Neisseria meningitidis; Oil-in-water emulsions, Aluminum salts; and Saponins, ISCOMs, the Seppic ISA series of Montanide adjuvants, Montanide ISA 720; PROVAX; Syntext Adjuvant Formulation; poly[di(carboxylatophenoxy) phosphazene and Leishmania elongation factor.

123. The pharmaceutical composition of claim 112, further comprising a therapeutic agent selected from the group consisting of an anti-viral agent, an anti-bacterial agent, an anti-parasitic agent, an anti-fungal agent, and a cancer medicament.

124. A method for treating an infectious disease in a subject, comprising: administering to a subject having an infectious disease a Th2 immunostimulatory nucleic acid in an amount effective to treat the infectious disease when administered mucosally, dermally, or parenterally, wherein the subject has not been exposed to a Th1 immunostimulatory nucleic acid.

125. The method of claim 124, wherein the infectious disease is not an extracellular infection.

126. The method of claim 124, wherein the method is a method for treating a viral infection.

127. The method of claim 126, further comprising, administering an anti-viral agent.

128. The method of claim 124, wherein the method is a method for treating or preventing a bacterial infection.

129. The method of claim 128, further comprising, administering an anti-bacterial agent.

130. The method of claim 124, wherein the method is a method for treating or preventing a parasitic infection.

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131. The method of claim 130, further comprising administering an anti-parasitic agent.

132. The method of claim 124, wherein the Th2 immunostimulatory nucleic acid is administered mucosally.

133. The method of claim 124, wherein the Th2 immunostimulatory nucleic acid is administered locally.

134. The method of claim 124, wherein the Th2 immunostimulatory nucleic acid is administered parenterally.

135. A method of preventing an infectious disease in a subject, comprising administering to a subject at risk of developing an infectious disease a Th2 immunostimulatory nucleic acid in an amount effective to prevent the infectious disease when administered mucosally, dermally, or parenterally, wherein the subject has not been exposed to a Th1 immunostimulatory nucleic acid.

136. A method for treating or preventing a cancer in a subject, comprising: administering to a subject having a cancer or at risk of developing a cancer a Th2 immunostimulatory nucleic acid in an amount effective to treat or prevent the cancer when administered mucosally, dermally, or parenterally.

137. The method of claim 136, wherein the cancer is a cancer selected from the group consisting of oral cavity cancer, throat cancer, stomach cancer, colon cancer, rectal cancer, cervical cancer.

138. The method of claim 136, wherein the Th2-immunostimulatory nucleic acid is administered mucosally.

139. The method of claim 136, wherein the Th2-immunostimulatory nucleic acid is administered locally.

140. The method of claim 136, wherein the Th2-immunostimulatory nucleic acid is administered parenterally.

141. The method of claim 136, further comprising administering an anti-cancer agent.

142. A method for stimulating an antibody dependent cellular cytotoxic (ADCC) immune response in a subject, comprising administering to the subject a Th2 immunostimulatory nucleic acid and an antibody in an effective amount for inducing ADCC.

143. The method of claim 142, wherein the antibody is a monoclonal antibody.

144. The method of claim 142, wherein the monoclonal antibody is selected from the group consisting of Rituxan, IDEC-C2B8, anti-CD20 Mab, Panorex, 3622W94, anti-EGP40 (17-1A) pancarcinoma antigen on adenocarcinomas Herceptin, anti-Her2, Anti-EGFr, BEC2, anti-idiotypic-GD.sub.3 epitope, Ovarex, B43.13, anti-idiotypic CA125, 4B5, Anti-VEGF, RhuMAb, MDX-210, anti-HER-2, MDX-22, MDX-220, MDX-447, MDX-260, anti-GD-2, Quadramet, CYT-424, IDEC-Y2B8, Oncolym, Lym-1, SMART M195, ATRAGEN, LDP-03, anti-CAMPATH, ior t6, anti CD6, MDX-11, OV103, Zenapax, Anti-Tac, anti-IL-2 receptor, MELIMMUNE-2, MELIMMUNE-1, CEACIDE, Pretarget, NovoMAb-G2, TNT, anti-histone, Gliomab-H, GNI-250, EMD-72000, LymphoCide, CMA 676, Monopharm-C, ior egf/r3, ior c5, anti-FLK-2, SMART 1D10, SMART ABL 364, and ImmuRAIT-CEA.

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145. The method of claim 142, wherein the subject has a disorder selected from the group consisting of cancer, and infectious disease.

146. The method of claim 142, wherein the Th2 immunostimulatory nucleic acid is not conjugated to the antibody.

147. The method of claim 142, wherein the subject has a cancer.

148. The method of claim 147, further comprising administering radiation or chemotherapy to the subject.

149. The method of claim 148, wherein the chemotherapy is selected from the group consisting of Taxol, cisplatin, doxorubicin, and adriamycin.

150. A pharmaceutical composition, comprising: a Th2 immunostimulatory nucleic acid in an effective amount for inducing ADCC, a monoclonal antibody, and a pharmaceutically acceptable carrier.

151. The composition of claim 150, wherein the monoclonal antibody is selected from the group consisting of Rituxan, IDEC-C2B8, anti-CD20 Mab, Panorex, 3622W94, anti-EGP40 (17-1A) pancarcinoma antigen on adenocarcinomas Herceptin, anti-Her2, Anti-EGFr, BEC2, anti-idiotypic-GD.sub.3 epitope, Ovarex, B43.13, anti-idiotypic CA125, 4B5, Anti-VEGF, RhuMAb, MDX-210, anti-HER-2, MDX-22, MDX-220, MDX-447, MDX-260, anti-GD-2, Quadramet, CYT-424, IDEC-Y2B8, Oncolym, Lym-1, SMART M195, ATRAGEN, LDP-03, anti-CAMPATH, ior t6, anti CD6, MDX-11, OV103, Zenapax, Anti-Tac, anti-IL-2 receptor, MELIMMUNE-2, MELIMMUNE-1, CEACIDE, Pretarget, NovoMAb-G2, TNT, anti-histone, Gliomab-H, GNI-250, EMD-72000, LymphoCide, CMA 676, Monopharm-C, ior egf/r3, ior c5, anti-FLK-2, SMART 1D10, SMART ABL 364, and ImmuRAIT-CEA.

152. A composition, comprising: a Th2 immunostimulatory nucleic acid having a phosphodiester backbone, formulated in a delivery vehicle selected from the group consisting of bioadhesive polymers, enteric-coated capsules, microspheres, nanospheres, and polymer rings.

153. The composition of claim 152, wherein the Th2 immunostimulatory nucleic acid is formulated for mucosal delivery.

Description

PRIORITY OF THE INVENTION

[0001] This application claims priority under Title 35 .sctn.119(e), of U.S. application Ser. No. 60/177,461, filed Jan. 20, 2000, entitled IMMUNOSTIMULATORY NUCLEIC ACIDS FOR INDUCING A TH2 RESPONSE, the entire contents of which are incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The invention relates to methods and products for inducing an immune response and preferably a Th2 immune response. In particular the invention relates to the use of immunostimulatory nucleic acids that preferentially induce a Th2 immune response. The invention is useful inter alia for treating and preventing disorders associated with a Th1 immune response or disorders that are sensitive to a Th2

immune response.

BACKGROUND OF THE INVENTION

[0003] The existence of functionally polarized T cell responses based on the profile of cytokines secreted by CD4+ T helper (Th) cells has been well established. In general, Th1 cells secrete interferon-gamma (IFN-.gamma.), interleukin (IL)-2, and tumor necrosis factor-beta (TNF.beta.), and are important in macrophage activation, the generation of both humoral and cell-mediated immune responses and phagocyte-dependent protective responses. Th2 cells secrete IL-4, IL-5, IL-10, and IL-13 and are more important in the generation of humoral immunity, eosinophil activation, regulation of cell-mediated immune responses, control of macrophage function and the stimulation of particular Ig isotypes (Morel et al., 1998, Romagnani, 1999). Th1 cells generally develop following infections by intracellular pathogens, whereas Th2 cells predominate in response to intestinal nematodes. In addition to their roles in protective immunity, Th1 and Th2 cells are responsible for different types of immunopathological disorders. For example, Th1 cells predominate in organ specific autoimmune disorders, Crohn's disease, Helicobacter pylori-induced peptic ulcer, acute solid organ allograft rejection, and unexplained recurrent abortion, whereas Th2 cells predominate in Omenn's syndrome, systemic lupus erythematosus, transplantation tolerance, chronic graft versus host disease, idiopathic pulmonary fibrosis, and progressive systemic sclerosis, and are involved in triggering of allergic reactions (Romagnani 1999, Singh et al., 1999). Therefore, for both prophylactic and therapeutic purposes, depending on the particular disease, a preference for either Th1 or Th2 type responses exists.

[0004] In recent years, a number of studies have demonstrated the ability of unmethylated CpG dinucleotides (i.e., the cytosine is unmethylated) within the context of certain flanking sequences (CpG motifs) to stimulate both innate and specific immune responses. Such sequences are commonly found in bacterial DNA which is immunostimulatory. Similar immunostimulation is also possible with synthetic oligodeoxynucleotides (ODN) containing CpG motifs (CpG ODN). It has been demonstrated that CpG DNA can induce stimulation of B cells to proliferate and secrete immunoglobulin (Ig), IL-6 and IL-12, and to be protected from apoptosis (Krieg et al., 1995, Yi et al., 1996, Klinman et al., 1996). These effects contribute to the ability of CpG DNA to have adjuvant activity. In addition, CpG DNA enhances expression of class II MHC and B7 co-stimulatory molecules (Davis et al., 1998, Sparwasser et al., 1998), that leads to improved antigen presentation. Furthermore, CpG DNA also directly activates monocytes, macrophages and dendritic cells to secrete various cytokines and chemokines (Klinman et al., 1996, Sparwasser et al., 1998, Halpern et al., 1996) that can provide T-helper functions. These in vitro effects were believed to be specific to the unmethylated CpG motifs since they were not induced by methylated bacterial DNA or in general by ODN that do not contain unmethylated CpG motifs.

[0005] Immunization of animals against a variety of antigens delivered both parenterally and mucosally demonstrate that addition of CpG ODN induces more Th1-like responses as indicated by strong cytotoxic T lymphocytes (CTL), high levels of IgG2a antibodies, and predominantly Th1 cytokines (e.g., IL-12 and IFN-.gamma. but not IL-4 or IL-5) (Klinman et al., 1996, Davis et al., 1998, Roman et al., 1997, Chu et al., 1997, Lipford et al., 1997, Weiner et al., 1997, McCluskie and Davis, 1998, 1999). In some circumstances, however, as outlined above, for immunization against certain diseases, a Th1 response is undesirable. For parenteral administration, aluminum precipitates (alum) may be added to antigens to augment Th2 immune responses, however alum is generally considered not suitable for delivery to mucosal surfaces. Cholera toxin (CT) is a potent Th2 mucosal adjuvant commonly used in animal models (Spangler 1992, Holmgren et al., 1992), however, it is considered to be too toxic for use in humans.

SUMMARY OF THE INVENTION

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[0006] The invention relates in some aspects to the discovery of compounds that induce a Th2 immune response. It has previously been demonstrated that oligonucleotides containing immunostimulatory CpG motifs (CpG ODN or CpG nucleic acids) are effective parenteral and mucosal adjuvants to protein antigens that induce Th1 immune responses. It has been discovered according to an aspect of the invention that oligonucleotides that do not contain immunostimulatory CpG motifs (non-CpG ODN), when administered by a mucosal route, augment immune responses and create a Th2 environment. The non-CpG ODN useful for producing these effects are referred to as Th2-immunostimulatory nucleic acids. These effects occur even with low doses of Th2 immunostimulatory nucleic acids. For instance, antibody levels are augmented almost as much as with CpG nucleic acids. While CpG nucleic acids push the immune responses in a Th1 direction, however, the Th2 immunostimulatory nucleic acids give a Th2-biased response. A "Th2 biased immune response" refers to the induction of at least one Th2-cytokine or an antibody typical of a Th2 response (Th2-antibody). This type of response was unexpected for several reasons. Th2 immunostimulatory nucleic acids do not induce this effect at typical adjuvant doses by parenteral routes. Nor do Th2 immunostimulatory nucleic acids have immune stimulatory effects in vitro that would predict such an in vivo response. It was also discovered that the Th2 immunostimulatory nucleic acids can produce an immune response such as an adjuvant effect with the administration of high doses by parenteral routes, or by direct delivery to affected tissues.

[0007] Thus one aspect of the invention is a method for inducing an antigen specific response by administering to a subject an antigen and a Th2-immunostimulatory nucleic acid in an amount effective to produce an antigen specific immune response when the Th2 immunostimulatory nucleic acid is administered mucosally or dermally. The effective amount is generally much lower than that required to induce an immune response when administered parenterally. Thus, in some embodiments, the effective dose ranges from 1 ng/kg to 1 mg/kg per administration. In other embodiments, the effective dose ranges from 0.01 .mu.g/kg to 500 .mu.g/kg per administration. In preferred embodiments, the range is from 0.1 .mu.g/kg to 250 .mu.g/kg per administration, in even more preferred embodiments, the range is from 1 .mu.g/kg to 100 .mu.g/kg per administration. In other embodiments, the mucosal or dermal effective amount ranges from 15 ng/kg to 150 .mu.g/kg per administration, and in still others from 150 ng/kg to 15 .mu.g/kg per administration. In some embodiments the Th2-immunostimulatory nucleic acid is delivered to the mucosa or locally to tissue such as the skin or eyeball. Although the Th2-immunostimulatory nucleic acid is administered mucosally or to the skin in some embodiments, it can produce a systemic immune response as well as a mucosal immune response. In certain embodiments, the dose of antigen administered along with the Th2 immunostimulatory nucleic acid is also lower than would be expected to be useful. In some embodiments doses of antigen which can effectively be used to induce an antigen specific immune response when administered with a Th2 immunostimulatory nucleic acid range from 0.1 .mu.g to 10 .mu.g total dose per administration, and in some instances from 1 .mu.g to 100 .mu.g total dose per administration. This range represents a 10-100 fold decrease over the amount of antigen which is required to induce an immune response when administered alone.

[0008] In another aspect of the invention, a method is provided for inducing an antigen specific response by administering to a subject an antigen and a Th2 immunostimulatory nucleic acid in an amount effective to produce an antigen specific immune response when the Th2 immunostimulatory nucleic acid is administered parenterally. The effective amount required for parenteral administration is greater than that which is effective for mucosal or dermal administration. Parenteral effective amounts range from 0.01 mg/kg to 1 mg/kg per administration, preferably when in a non-formulated form. If the Th2 immunostimulatory nucleic acids are formulated, and especially when they are formulated together with an antigen, the doses can be reduced in some instances to as low as 0.0001 mg/kg per administration. The immune response generated in this manner is a systemic immune response.

[0009] In the most preferred embodiments, the Th2 immunostimulatory nucleic acids are administered at doses not exceeding 1 mg/kg per administration, whether delivered mucosally or parenterally.

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[0010] In certain embodiments of the foregoing aspects, the antigen is not conjugated to the Th2 immunostimulatory nucleic acid. In important embodiments, the antigen is not a self antigen, and it is not bacterial or a viral antigen.

[0011] According to another aspect of the invention a method for treating a non-autoimmune Th1-mediated disease in a subject is provided. The method includes administering to a subject a Th2-immunostimulatory nucleic acid in an amount effective to produce a Th2 immune response, when the Th2 immunostimulatory nucleic acid is administered mucosally or dermally.

[0012] Another aspect of the invention provides a method for treating autoimmune disease is a subject. The method comprises administering to a subject a Th2 immunostimulatory nucleic acid in an amount effective to produce a Th2 immune response, when the Th2 immunostimulatory nucleic acid is administered mucosally or dermally. In some embodiments the method also involves administering an antigen, such as, for instance a self-antigen, to the subject, for instance, to produce an immune hyporesponsive state. In important embodiments particularly those involving the treatment of Th1 mediated autoimmune disease, if the antigen is a self antigen, the antigen and Th2 immunostimulatory nucleic acid are not conjugated to each other.

[0013] Importantly, in some embodiments, the subject has not been exposed to a Th1 immunostimulatory nucleic acid. As an example, the subject in some embodiments, has not been exposed to a bacteria or a virus that carries a Th1 immunostimulatory nucleic acid. The subject may have been exposed to a parasite, such an extracellular parasite or an obligate intracellular parasite. Thus, in some embodiments, the subject does not have a bacterial or viral infection. In several aspects of the invention, the subject is not experiencing an immune response that is attributable to a Th1 immunostimulatory nucleic acid. Rather, in certain aspects, the subject is not experiencing an immune response attributable to a Th1 immunostimulatory nucleic acid because the subject has not been in contact with a Th1 immunostimulatory nucleic acid.

[0014] In other embodiments, the subject is administered a Th1 immunostimulatory nucleic acid following the administration of the Th2 immunostimulatory nucleic acid. In still other embodiments, the Th2 immunostimulatory nucleic acid is administered to a subject at risk of developing an extracellular infection. In important embodiments, the extracellular infections include those that colonize mucosal tissues and surfaces such as fungal and yeast infections that are sexually transmitted or that affect cancer patients receiving chemotherapy.

[0015] The T2 immunostimulatory nucleic acids may comprise phosphodiester or a phosphorothioate backbone. Importantly, immunization at the mucosal surface is not dependent upon backbone modification, and phosphodiester backbone nucleic acids are as effective as phosphorothioate backbone modifications for inducing an immune response. This is a surprising finding given that phosphorothioate backbone nucleic acids have been reported to be more efficient as parenterally administered vaccines.

[0016] The Th2 immune response induced according to the methods of the invention is not dependent upon conjugation of antigen and the Th2 immunostimulatory nucleic acid. Thus, the antigen and the nucleic acid may be conjugated to each other but this is not required. In some embodiments, it is preferred that the antigen and nucleic acid are not conjugated to each other. Thus, the antigen and the Th2-immunostimulatory nucleic acid may be administered simultaneously or separately. For instance, the antigen may be administered after the Th2-immunostimulatory nucleic acid or before the Th2-immunostimulatory nucleic acid. Additionally, the antigen and the Th2-immunostimulatory nucleic acid may be administered to the same or different sites in the subject and may be administered using the same or different delivery vehicles. For instance, in some embodiments the antigen is delivered to the mucosa

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or skin and in other embodiments the antigen is administered parenterally. In important embodiments, antigens may be administered in low doses, or alternatively, antigens with low antigenicity or immunogenicity may be used in the methods of the invention. Administration of low doses of antigen with a Th2 immunostimulatory nucleic acid, particularly when administered mucosally, surprisingly results in a Th2 immune response against the antigen, rather than a Th1 antigen specific immune response or antigen specific tolerance, both of which have been reported following low dose antigen administration. Antigens reported to have poor immunogenicity profiles include peptide antigens and tumor antigens. Additionally, the methods of the invention can be used to stimulate an immune response in subjects who are hyporesponsive to a particular antigen, such as for example, Hepatitis B surface antigen.

[0017] In some embodiments the method also includes administering a therapeutic agent to the subject. The therapeutic agent in some embodiments is a Th1 adjuvant, a Th2 adjuvant, a cytokine, and/or a drug for treating Th1 mediated disorders, such as, for instance an anti-psoriasis cream.

[0018] The Th2-immunostimulatory nucleic acid and/or antigen and/or therapeutic agent may be formulated and delivered to the subject in any manner known in the art. For instance in some embodiments it is formulated in a liquid solution, as a powder or in a bioadhesive polymer. In other embodiments the Th2-immunostimulatory nucleic acid is administered to the skin or a superficially located mucosal membrane using a needleless jet injection or particulate delivery system, scarification, and/or tines. In yet other embodiments the antigen and/or therapeutic agent is administered using a delivery system selected from the group consisting of a needleless delivery system, a scarification delivery system, and a tine delivery system.

[0019] In some aspects of the invention, the Th2-immunostimulatory nucleic acid is administered to the mucosa or skin. In some embodiments the Th2-immunostimulatory nucleic acid is administered orally, intranasally, by inhalation, rectally, vaginally, intradermally, intra-ocularly, intraepidermally, or transdermally.

[0020] In some embodiments of the invention the method is a method for treating or preventing a Th1 mediated disorder. The Th1 mediated disorder may be selected from the group consisting of an autoimmune disease, Helicobacter pylori-induced peptic ulcer, psoriasis, Th1 inflammatory disorder (provided it is not induced by the presence of bacterial or viral Th1 immunostimulatory nucleic acid), acute kidney allograft rejection, and unexplained recurrent abortion. The autoimmune disease in other embodiments is selected from the group consisting of rheumatoid arthritis, Crohn's disease, multiple sclerosis, systemic lupus erythematosus, autoimmune encephalomyelitis, myasthenia gravis, and insulin-dependent diabetes.

[0021] According to other embodiments the method is a method for inducing a local Th2 environment in the subject. The subject may have, for instance, a Th1 mediated skin disorder, and the local Th2 environment is induced in the skin.

[0022] The invention in other aspects relates to pharmaceutical compositions. One pharmaceutical composition of the invention includes a Th2-immunostimulatory nucleic acid and an antigen in a pharmaceutically acceptable carrier. The composition may optionally include a therapeutic agent.

[0023] Yet another pharmaceutical composition includes a Th2-immunostimulatory nucleic acid and an adjuvant, in a pharmaceutically acceptable carrier. This composition may also optionally include an antigen.

[0024] The Th2-immunostimulatory nucleic acid and/or the antigen and/or therapeutic agent are in some

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embodiments formulated together or separately in a delivery vehicle selected from the group consisting of bioadhesive polymers, cochleates, dendrimers, enteric-coated capsules, emulsomes, ISCOMs, liposomes, microspheres, nanospheres, polymer rings, proteosomes, and virosomes. In some embodiments the Th2-immunostimulatory nucleic acid and antigen and/or therapeutic agent are present in different delivery vehicles and in other embodiments they are in the same delivery vehicles.

[0025] When the composition or methods include a therapeutic agent, the therapeutic agent may be, in some embodiments, a Th1 adjuvant, a Th2 adjuvant, a cytokine, an anti-bacterial agent, an anti-fungal agent, an anti-parasitic agent, an anti-viral agent, or a drug for treating Th1 mediated disorders.

[0026] In some embodiments the Th1 adjuvant is a CpG nucleic acids, MF59, SAF, MPL, or QS21. In other embodiments the Th2 adjuvant is selected from the group consisting of adjuvants that creates a depot effect, adjuvants that stimulate the immune system, adjuvants that create a depot effect and stimulate the immune system and mucosal adjuvants. Adjuvants that creates a depot effect include but are not limited to alum; emulsion-based formulations including mineral oil, non-mineral oil, water-in-oil or oil-in-water-in oil emulsion, oil-in-water emulsions such as Seppic ISA series of Montanide adjuvants; and PROVAX. Adjuvants that stimulates the immune system include but are not limited to saponins purified from the bark of the Q. saponaria tree; poly[di(carboxylatopheno- xy)phosphazene; derivatives of lipopolysaccharides, muramyl dipeptide and threonyl-muramyl dipeptide; OM-174; and Leishmania elongation factor. Adjuvants that create a depot effect and stimulate the immune system include but are not limited to ISCOMs; SB-AS2; SB-AS4; non-ionic block copolymers that form micelles such as CRL 1005; and Syntex Adjuvant Formulation.

[0027] Mucosal adjuvants include but are not limited to CpG nucleic acids, Bacterial toxins, Cholera toxin, CT derivatives, CT B subunit; CTD53; CTK97; CTK104; CTD53/K63; CTH54; CTN107; CTE114; CTE112K; CTS61F; CTS106; and CTK63, Zonula occludens toxin, zot, Escherichia coli heat-labile enterotoxin, Labile Toxin, LT derivatives, LT B subunit; LT7K; LT61F; LT112K; LT118E; LT146E; LT192G; LTK63; and LTR72, Pertussis toxin, PT-9K/129G; Toxin derivatives; Lipid A derivatives, MDP derivatives; Bacterial outer membrane proteins, outer surface protein A (OspA) lipoprotein of Borrelia burgdorferi, outer membrane protein of Neisseria meningitidis; Oil-in-water emulsions, Aluminum salts; and Saponins, ISCOMs, the Seppic ISA series of Montanide adjuvants, Montanide ISA 720; PROVAX; Syntext Adjuvant Formulation; poly[di(carboxylatophenoxy) phosphazene and Leishmania elongation factor.

[0028] Drugs for treating Th1 mediated disorders include but are not limited to anti-psoriasis creams, eye drops, nose drops, sulfasalazine, glucocorticoids, propylthiouracil, methimazole, .sup.131I, insulin, IFN-.beta.1a, IFN-.beta.1b, copolymer 1 (i.e., MS), glucocorticoids (i.e., MS), ACTH, avonex, azathioprine, cyclophosphamide, UV-B, PUVA, methotrexate, calcipitriol, cyclophosphamide, OKT3, FK-506, cyclosporin A, azathioprine, and mycophenolate mofetil.

[0029] The invention in other aspects relates to an improved method of the type involving antigen dependent cellular cytotoxicity (ADCC) for stimulating an immune response in a subject. The improvement in the method involves administering to the subject a Th2 immunostimulatory nucleic acid in an effective amount for inducing ADCC. In some embodiments the subject has cancer or is at risk of developing cancer. In some embodiments a monoclonal antibody is also administered to the subject. Monoclonal antibodies include but are not limited to Rituxan, IDEC-C2B8, anti-CD20 Mab, Panorex, 3622W94, anti-EGP40 (17-1A) pancarcinoma antigen on adenocarcinomas Herceptin, anti-Her2, Anti-EGFr, BEC2, anti-idiotypic-GD.sub.3 epitope, Ovarex, B43.13, anti-idiotypic CA125, 4B5, Anti-VEGF, RhuMAb, MDX-210, anti-HER-2, MDX-22, MDX-220, MDX-447, MDX-260, anti-GD-2, Quadramet, CYT-424, IDEC-Y2B8, Oncolym, Lym-1, SMART M195, ATRAGEN, LDP-03, anti-CAMPATH, ior t6, anti CD6, MDX-11, OV103, Zenapax, Anti-Tac, anti-IL-2 receptor, MELIMMUNE-2,

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MELIMMUNE-1, CEACIDE, Pretarget, NovoMAb-G2, TNT, anti-histone, Gliomab-H, GNI-250, EMD-72000, LymphoCide, CMA 676, Monopharm-C, ior egf/r3, ior c5, anti-FLK-2, SMART 1D10, SMART ABL 364, and ImmuRAIT-CEA.

[0030] In other embodiments radiation or chemotherapy is administered to the subject. Chemotherapies include but are not limited to Taxol, cisplatin, doxorubicin, and adriamycin.

[0031] The invention in other aspects is a pharmaceutical composition of a Th2 immunostimulatory nucleic acid in an effective amount for inducing ADCC and a monoclonal antibody. Monoclonal antibodies include but are not limited to Rituxan, IDEC-C2B8, anti-CD20 Mab, Panorex, 3622W94, anti-EGP40 (17-1A) pancarcinoma antigen on adenocarcinomas Herceptin, anti-Her2, Anti-EGFr, BEC2, anti-idiotypic-GD.sub.3 epitope, Ovarex, B43.13, anti-idiotypic CA125, 4B5, Anti-VEGF, RhuMAb, MDX-210, anti-HER-2, MDX-22, MDX-220, MDX-447, MDX-260, anti-GD-2, Quadramet, CYT-424, IDEC-Y2B8, Oncolym, Lym-1, SMART M195, ATRAGEN, LDP-03, anti-CAMPATH, ior t6, anti CD6, MDX-11, OV103, Zenapax, Anti-Tac, anti-IL-2 receptor, MELIMMUNE-2, MELIMMUNE-1, CEACIDE, Pretarget, NovoMAb-G2, TNT, anti-histone, Gliomab-H, GNI-250, EMD-72000, LymphoCide, CMA 676, Monopharm-C, ior egf/r3, ior c5, anti-FLK-2, SMART 1D10, SMART ABL 364, and ImmuRAIT-CEA.

[0032] According to other aspects, the invention relates to a composition of a Th2 immunostimulatory nucleic acid having a phosphodiester backbone, formulated in a delivery vehicle selected from the group consisting of bioadhesive polymers, enteric-coated capsules, microspheres, nanospheres, and polymer rings. In important embodiments, the phosphodiester Th2 immunostimulatory nucleic acid is formulated for mucosal delivery.

[0033] Each of the limitations of the invention can encompass various embodiments of the invention. It is therefore anticipated that each of the limitations of the invention involving any one element or combination of elements can be included in each aspect of the invention.

BRIEF DESCRIPTION OF THE SEQUENCE LISTING

[0034] SEQ ID NO: 1 is the nucleotide sequence of non-CpG ODN #1982.

[0035] SEQ ID NO: 2 is the nucleotide sequence of non-CpG ODN #2138.

[0036] SEQ ID NO: 3 is the nucleotide sequence of CpG ODN #1826.

[0037] SEQ ID NO: 4 is the nucleotide sequence of CpG ODN #2006.

BRIEF DESCRIPTION OF THE DRAWINGS

[0038] FIG. 1 is a bar graph depicting the effect of different oligonucleotides on HBsAg-specific IgG titers. FIGS. 1a and 1b show data from an ELISA end-point dilution titer for HBsAg-specific antibodies (anti-HBs GMT) in plasma taken 1 week after final oral immunization (on days 0, 7 and 14) with HBsAg (100 .mu.g) without adjuvant or in combination with CpG ODN (motif#1826, 100 .mu.g), non-CpG ODN (motif#1982, 100 or 500 .mu.g) or Cholera toxin (CT, 10 .mu.g) for total IgG (FIG. 1a) or IgG1 (black bars) and IgG2a (hatched bars) isotypes (FIG. 1b).

[0039] FIG. 2 is a bar graph depicting the effect of different oligonucleotides on HBsAg-specific IgG titers. BALB/c mice were immunized by intramuscular (IM) injection with 1 .mu.g HBsAg without adjuvant or with 10 .mu.g of CpG ODN (motif #1826) or non-CpG ODN (motif #1982) and the ELISA

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end-point dilution titer for HBsAg-specific antibodies (anti-HBs), total IgG (FIG. 2a) or IgG1 (hatched bars) or IgG2a (grey bars) isotypes (FIG. 2b), in plasma taken 4 weeks after immunization is shown.

[0040] FIG. 3 is a bar graph depicting the effect of different oligonucleotides on TT-specific IgG titers. BALB/c mice were immunized by oral delivery on days 0, 7 and 14 with TT (100 .mu.g) without adjuvant or in combination with CpG ODN (motif #1826, 100 .mu.g), non-CpG ODN (motif#41982, 100 or 500 .mu.g) or Cholera toxin (CT, 10 .mu.g) and the ELISA end-point dilution titer for TT-specific antibodies (anti-TT GMT), total IgG (FIG. 3a) or IgG1 (hatched bars) or IgG2a (grey bars) isotypes (FIG. 3b), in plasma taken 1 week after final immunization are shown.

[0041] FIG. 4 is a bar graph depicting the effect of different oligonucleotides on FLUVIRAL.RTM.-specific IgG titers. BALB/c mice were immunized by oral delivery on days 0, 7 and 14 with FLUVIRAL.RTM. (50 .mu.l, {fraction (1/10)} human dose) without adjuvant or in combination with 10 .mu.g of CpG ODN (motif #1826) or non-CpG ODN (motif #2138 or #1982) and the ELISA end-point dilution titer for FLUVIRAL.RTM.-specific antibodies (anti-FLUVIRAL.RTM. GMT), total IgG (FIG. 4a) or IgG1 (hatched bars) or IgG2a (grey bars) isotypes (FIG. 4b), in plasma taken 1 week after final immunization are shown.

[0042] FIG. 5 is a bar graph showing the effect of different oligonucleotides on FLUARIX.RTM.-specific IgG titers. BALB/c mice were immunized by intramuscular (IM) injection with FLUARIX.RTM. (50 .mu.l, {fraction (1/10)} human dose) without adjuvant or in combination with 50 .mu.g of CpG ODN (motif #2006) or non-CpG ODN (motif #1982) and the ELISA end-point dilution titer for FLUARIX-specific antibodies (anti-FLUARIX.RTM.) in plasma taken 2 weeks after immunization is shown.

[0043] FIG. 6 is a graph depicting the effect of different oligonucleotides on antigen-specific IgG titers. BALB/c mice were immunized by oral delivery on days 0, 7 and 14 with a combination of HBsAg/TT/FLUVIRAL.RTM. (10 .mu.g, 10 .mu.g, 50 .mu.l respectively) without adjuvant or in combination with 10 .mu.g CpG ODN (motif #1826), or non-CpG ODN (motif #1982) and the ELISA end-point dilution titer for HBsAg-specific antibodies (FIG. 6a), TT-specific antibodies (FIG. 6b, HBsAg/TT/FLUVIRAL.RTM., filled circles or single antigen TT, filled triangles), FLUVIRAL-specific antibodies (FIG. 6c, HBsAg/TT/FLUVIRAL.RTM., filled circles or with a single antigen FLUVIRAL.RTM., filled triangles) in plasma of individual mice taken 1 week after final immunization is shown. Other mice were immunized with TT or FLUVIRAL.RTM. with 10 .mu.g CpG ODN (motif #1826). Horizontal bars represent the group geometric mean.

[0044] FIG. 7 is a graph depicting the effect of different oligonucleotides on antigen-specific IgG titers. BALB/c mice were immunized by oral delivery on days 0, 7 and 14 with a combination of HBsAg/TT/FLUVIRAL.RTM. (10 .mu.g, 10 .mu.g, 50 .mu.l respectively) without adjuvant or in combination with 10 .mu.g CpG ODN (motif #1826), or non-CpG ODN (motif #1982) and the ELISA end-point dilution titer for FLUVIRAL.RTM.-specific (FIG. 7a) or TT-specific (FIG. 7b) antibodies of IgG1 (grey bars) or IgG2a (black bars) isotypes in plasma taken 1 week after final immunization is shown.

[0045] FIG. 8 is a bar graph depicting the effect of different oligonucleotides on TT-specific IgG titers. BALB/c mice were immunized by intrarectal (FIG. 8a), intranasal (FIG. 8b), or oral (FIG. 8c) delivery on days 0, 7 and 14 with TT (10 .mu.g) without adjuvant or in combination with CpG ODN (motif #1826, 100 .mu.g), non-CpG ODN (motif #1982, 100 .mu.g) or Cholera toxin (CT, 10 .mu.g) and the ELISA end-point dilution titer for TT-specific antibodies in plasma of individual mice taken 1 week after final immunization is shown.

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[0046] FIG. 9 is a bar graph depicting the effect of different oligonucleotides by intranasal delivery on TT-specific IgG titers. BALB/c mice were immunized by intranasal delivery on days 0, 7 and 14 with TT (10 .mu.g) without adjuvant or in combination with CpG ODN (motif #1826, 10 or 100 .mu.g) or non-CpG ODN (motif #1982, 100 .mu.g) and the ELISA end-point dilution titer for TT-specific antibodies (anti-TT GMT), total IgG (FIG. 9a) or of IgG1 (grey bars) or IgG2a (hatched bars) isotypes (FIG. 9b) in plasma taken 1 week after final immunization is shown.

[0047] FIG. 10 is a bar graph depicting the effect of different oligonucleotides by oral delivery on TT-specific IgG titers. BALB/c mice were immunized by oral delivery on days 0, 7 and 14 with TT (10 .mu.g) without adjuvant or in combination with CpG ODN (motif #1826, 10 or 100 .mu.g) or non-CpG ODN (motif #1982, 10 or 100 .mu.g) and the ELISA end-point dilution titer for TT-specific antibodies (anti-TT GMT) total IgG (FIG. 10a) or IgG1 (grey bars) or IgG2a (hatched bars) isotypes (FIG. 10b) in plasma taken 1 week after final immunization. Titers were defined as the highest plasma dilution resulting in an absorbance value two times that of non-immune plasma, with a cut-off value of 0.05.

[0048] FIG. 11 is a bar graph depicting the effect of different oligonucleotides on HBsAg-specific IgA titers. BALB/c mice were immunized by oral delivery on days 0, 7 and 14 with HBsAg (100 .mu.g) without adjuvant or in combination with CpG ODN (motif #1826, 100 or 500 .mu.g), or non-CpG ODN (motif #1982, 100 or 500 .mu.g) and the ELISA end-point dilution titer for HBsAg-specific IgA antibodies (anti-HBs IgA) in saliva (FIG. 11a), vaginal washes (FIG. 11b) and lung washes (FIG. 11c) taken 1 week after final immunization and pooled for each group are shown.

[0049] FIG. 12 is a bar graph depicting the effect of different oligonucleotides on TT-specific IgA titers. BALB/c mice were immunized by oral delivery on days 0, 7 and 14 with TT (100 .mu.g) without adjuvant or in combination with CpG ODN (motif #1826, 100 or 500 .mu.g), non-CpG ODN (motif #1982, 100 or 500 .mu.g) or Cholera toxin (CT, 10 .mu.g) and the ELISA end-point dilution titer for TT-specific IgA antibodies (anti-TT IgA) in vaginal washes collected 1 week after final immunization and pooled for each group is shown.

[0050] FIG. 13 is a bar graph depicting the effect of different oligonucleotides on FLUVIRAL.RTM.-specific IgA titers. BALB/c mice were immunized by oral delivery on days 0, 7 and 14 with FLUVIRAL.RTM. (50 .mu.l, {fraction (1/10)} human dose) without adjuvant or in combination with 10 .mu.g of CpG ODN (motif #1826) or non-CpG ODN (motif #2138) and the ELISA end-point dilution titer for FLUVIRAL.RTM.-specific IgA antibodies (anti-FLUVIRAL.RTM. IgA) for individual mice in lung washes (FIG. 13a), vaginal washes (FIG. 13b), and saliva (FIG. 13c) taken 1 week after final immunization is shown.

[0051] FIG. 14 is a graph depicting the effect of different oligonucleotides on antigen-specific IgA titers. BALB/c mice were immunized by oral delivery on days 0, 7 and 14 with a combination of HBsAg/TT/FLUVIRAL.RTM. (10 .mu.g, 10 .mu.g, 50 .mu.l respectively) without adjuvant or in combination with 10 .mu.g CpG ODN (motif #1826), or non-CpG ODN (motif #1982) and the ELISA end-point dilution titer for TT-specific IgA antibodies (FIG. 14a), HBsAg-specific IgA antibodies (FIG. 14b), and FLUVIRAL.RTM.-specific IgA antibodies in lung washes of individual mice taken 1 week after final immunization is shown.

DETAILED DESCRIPTION OF THE INVENTION

[0052] The invention is based in part on the discovery that certain nucleic acid molecules, when administered to a subject, induce a Th2 biased immune response. It was previously known in the art that CpG containing nucleic acids produce a Th1 immune response, but it was believed that nucleic acids

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lacking a CpG do not produce an immune response. Surprisingly, it was discovered that control oligonucleotides, nucleic acids that do not include a CpG, actually do produce an immune response when administered in vivo but that the type of immune response differs from that produced by CpG containing nucleic acids.

[0053] As shown in the Examples below, mice were immunized by intramuscular (IM), oral, intranasal (IN) or intrarectal (IR) administration of one of three antigens: purified small envelope protein of the hepatitis B virus (S protein), which comprises hepatitis B surface antigen (HBsAg); tetanus toxoid (TT); or an influenza virus vaccine (FLUVIRAL.RTM.). Single or multiple antigen combinations were used either alone or with CpG nucleic acids or Th2 immunostimulatory nucleic acids as adjuvant. As shown previously, CpG nucleic acids augmented antigen-specific antibody responses with all routes, and this gave a much more Th1-biased response than was obtained with antigen alone. As also shown previously, non-CpG nucleic acids had no effect when given by a parenteral route (e.g., intramuscularly, IM) at normal parenteral doses. Antibody responses were essentially the same as those with antigen alone at these doses. However, surprisingly, when administered by any of the mucosal routes (including low dose administration) or at high doses through parenteral routes, the Th2 immunostimulatory nucleic acids did augment antibody responses, often as much as did the CpG nucleic acids, however the response was Th2-biased (IgG1>>IgG2a). This was particularly unexpected since in vitro data do not predict an immunostimulatory role for these Th2 immunostimulatory nucleic acids. This discovery has important implications for induction of immune responses where Th1 -type responses are undesirable or Th2-type responses are essential, and in the treatment of Th1 -associated disorders, as well as generally in the induction of antigen specific immune responses. Additionally, the invention provides methods for inducing mucosal immune responses, and systemic immune responses, particularly to antigens that are administered in low dose or which have a low immunogenicity.

[0054] The methods of the invention are intended for a wide range of subjects. The Th2 immunostimulatory nucleic acids are effective in subjects when used prophylactically or therapeutically. Additionally, the Th2 immunostimulatory nucleic acids are effective in subjects who have not been previously exposed to Th1 immunostimulatory nucleic acids. A subset of subjects having a bacterial or viral infection have been exposed to a Th1 immunostimulatory nucleic acid derived from the infecting bacteria or virus. Thus, the efficacy of the Th2 immunostimulatory nucleic acids in the methods of the invention are not dependent upon the presence of Th1 immunostimulatory nucleic acids. In some aspects, the invention intends that the Th2 immunostimulatory nucleic acids be used in the treatment of Th1 mediated disorders which are not associated with the presence of Th1 immunostimulatory nucleic acids, especially Th1 immunostimulatory nucleic acids derived from bacteria and viruses.

[0055] In other aspects of the invention, the Th2 immunostimulatory nucleic acids are not intended to reduce a pre-existing a Th1 immune response, but rather are intended to induce a Th2 immune response, irrespective of a down-regulation of a Th1 immune response. Some Th2 immunostimulatory nucleic acids are capable of inducing some level of Th1 immune response, thus in some instances, administration of a Th2 immunostimulatory nucleic acid will result in an up-regulation of both a Th2 and a Th1 immune response, albeit with a bias towards the Th2 immune response. It should be understood that in these latter instances administration of the Th2 immunostimulatory nucleic acids will result in increase and not decrease in the level of Th1 antibodies and cytokines over pre-administration levels.

[0056] Many of the methods provided by the invention involve mucosal or dermal administration of Th2 immunostimulatory nucleic acids at doses that have no effect when administered parenterally (e.g., intramuscularly, intravenously, intraperitoneally, subcutaneously, or by infusion). Other methods of the invention are capable of inducing Th2 immune responses when the Th2 immunostimulatory nucleic acids are administered parenterally at high doses. Thus, as used herein, the term "effective amount" is

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dependent upon the route of administration, with effective mucosal or dermal amounts being much lower than parenteral effective amounts.

[0057] Thus, in one aspect the invention is a method for inducing an antigen specific response by administering to a subject an antigen and a Th2-immunostimulatory nucleic acid in an amount effective to produce an antigen specific immune response.

[0058] The results of the experiments presented in the Examples show that Th2 immunostimulatory nucleic acids act as an effective adjuvant to induce immune responses against two different protein antigens (HBsAg, TT) as well as a killed split viral vaccine (FLUVIRAL.RTM.) when administered at typical adjuvant doses to the mucosal surfaces of the respiratory or gastrointestinal tracts. This effect was totally unexpected since non-CpG nucleic acids do not have such an effect when they are delivered by a parenteral route (e.g., IM injection) in amounts normally sufficient for CpG nucleic acids to induce an immune response (Davis et al., 1998), nor do they cause innate immune activation when added in vitro to cultures of peripheral blood mononuclear cells (Krieg et al., 1995). The Th2 immunostimulatory nucleic acids when administered to the mucosa were able to induce levels of antigen-specific IgG in the plasma as much as did CpG nucleic acids. Both nucleic acids were also as effective as CT, a strong conventional mucosal adjuvant that is highly effective in mice but too toxic for human use. Mucosal delivery of vaccines is particularly attractive since it offers: ease, low cost and safety of administration (e.g., orally, nasal drops or spray, inhalation, intrarectal, intravaginal or ocular administrations), thus removing the need for syringes and highly trained personnel; the generation of protective immunity at sites distant from the immunization site (Haneberg et al., 1994, Gallichan et al., 1995); no risk of needle stick injury or cross contamination through repeated use of the same needle, for example in poorer areas of the world; and, a broader age range of recipients (Walker et al., 1994).

[0059] Additionally, it was discovered that high doses of Th2 immunostimulatory nucleic acids administered in vivo are capable of provoking an immune response. This is surprising because it has been reported extensively in the literature that CpG nucleic acids induce an immune response through the presence of unmethylated CpG dinucleotides. Control nucleic acids without CpG motifs (i.e., lacking CpG dinucleotides or having CpG in which the C is methylated) have failed to produce immune responses at the doses tested. As a result, the investigators have concluded that the unmethylated CpG dinucleotide is essential. Additionally, in vitro studies using control nucleic acids have indicated that the unmethylated CpG was essential to the ability of the nucleic acid to induce an immune response. It has been discovered that high doses of non-CpG containing nucleic acids when administered in vivo have antigen-specific immune stimulating properties.

[0060] A "Th2 immunostimulatory nucleic acid" as used herein is a nucleic acid that does not contain an unmethylated CpG dinucleotide and that produces a Th2 immune response. An unmethylated CpG dinucleotide refers to an unmethylated cytosine within the dinucleotide. Thus, the Th2 immunostimulatory nucleic acid may be a nucleic acid that does not have any CpG dinucleotides. Additionally, the Th2 immunostimulatory nucleic acid is not T-rich or does not contain a poly T motif (i.e., a TTTT motif), a poly G motif (i.e., a GGGG motif), or a methylated CpG motif.

[0061] The Th2 immunostimulatory nucleic acids produce an immune response that is predominately Th2 in nature. A "Th2 immune response" as used herein refers to the induction of at least one Th2 cytokine or antibody typical of a Th2 response (Th2 antibody). In some embodiments more than one Th2-cytokine or Th2-antibody is induced, optionally in the absence of CTL, which are associated with Th1 responses. Thus the ability of a nucleic acid to produce a Th2 immune response can be assessed by determining if a Th2-cytokine or Th2-antibody is induced. This can be accomplished using routine screening. For instance, test nucleic acids can be administered alone or with antigen to mice or other animals, e.g., orally, and then the mouse or other animal can be screened for any changes in cytokine or

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antibody profiles. Some Th2 immunostimulatory nucleic acids are also capable of inducing a Th1 immune response, albeit at lower levels than the Th2 immune response induced.

[0062] Thus the induction of a Th2 response refers to the partial or complete induction of at least one Th2-cytokine or Th2-antibody or an increase in the levels of at least one Th2-cytokine or Th2-antibody. The term "cytokine" is used as a generic name for a diverse group of soluble proteins, factors, co-stimulatory molecules, and peptides which act as humoral regulators at nano- to picomolar concentrations and which, either under normal or pathological conditions, modulate the functional activities of individual cells and tissues. These cytokines also mediate interactions between cells directly and regulate processes taking place in the extracellular environment. Cytokines play a role in directing the T cell response. Helper (CD4+) T cells orchestrate the immune response of mammals through production of soluble factors that act on other immune system cells, including other T cells. Most mature CD4+ T helper cells express one of two cytokine profiles: Th1 or Th2. Examples of cytokines secreted by T cells or other immune cells that are associated with Th1 responses include IL-2, IL-12, IL-13, interferon-.gamma. (.gamma.-IFN), and TNF.beta.. The Th1 subset promotes delayed-type hypersensitivity, cell-mediated immunity, and immunoglobulin class switching to IgG.sub.2a. The Th2 subset induces humoral immunity by activating B cells, promoting antibody production, and inducing class switching to IgG.sub.1 and IgE. Examples of Th2 cytokines include, but are not limited to IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13. Th2-antibodies include but are not limited to IgG1 and IgE. Preferably the amount of Th2 antibodies generated by the Th2 immunostimulatory nucleic acids is the same or greater than the amount of Th1 antibodies generated. Some Th1 antibodies, such as IgG2a, may also be induced, but they will not be the predominant form of antibody.

[0063] The Th2 immunostimulatory nucleic acids can be double-stranded or single-stranded. Generally, double-stranded molecules are more stable in vivo, while single-stranded molecules have increased immune stimulating activity.

[0064] Th1 immunostimulatory nucleic acids, as used herein, refer to nucleic acids that induce primarily a Th1 immune response. Examples of Th1 immunostimulatory nucleic acids include nucleic acids containing at least one unmethylated CpG motif and/or nucleic acids that are T-rich. Th1 immunostimulatory nucleic acids are associated with some bacterial and viral strains. Infection by these microbes induces a Th1 immune response. A Th1 immune response is an immune response characterized by one or more Th1 cytokines or Th1 antibodies, as described herein.

[0065] The terms "nucleic acid" and "oligonucleotide" are used herein to mean multiple nucleotides (i.e. molecules comprising a sugar (e.g. ribose or deoxyribose) linked to a phosphate group and to an exchangeable organic base, which is either a substituted pyrimidine (e.g. cytosine (C), thymine (T) or uracil (U)) or a substituted purine (e.g. adenine (A) or guanine (G)). Substituted pyrimidines and purines include both naturally occurring and synthetic bases. As used herein, the terms refer to oligoribonucleotides as well as oligodeoxyribonucleotides. The terms shall also include polynucleosides (i.e. a polynucleotide minus the phosphate) and any other organic base containing polymer. Nucleic acid molecules can be obtained from existing nucleic acid sources (e.g. genomic or cDNA), but are preferably synthetic (e.g. produced by oligonucleotide synthesis).

[0066] The term Th2 immunostimulatory nucleic acid, however, does not encompass a plasmid expression vector. As used herein the terms a "Th2 immunostimulatory nucleic acid or oligonucleotide" and a "plasmid expression vector" are mutually exclusive. The terms "Th2 immunostimulatory nucleic acid or oligonucleotide" are used to refer to any Th2 immunostimulatory nucleic acid except for an expression vector. An expression vector as used herein is a nucleic acid molecule which includes at least a promoter and a gene encoding a peptide or peptide fragment and which is capable of expressing the peptide or peptide fragment in a cell. The plasmid expression vector includes a nucleic acid sequence

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encoding the peptide which is operatively linked to a gene expression sequence which directs the expression of the peptide within a eukaryotic cell. The gene expression sequence is any regulatory nucleotide sequence, such as a promoter sequence or promoter-enhancer combination, which facilitates the efficient transcription and translation of the peptide to which it is operatively linked. The gene expression sequence may, for example, be a mammalian or viral promoter, such as a constitutive or inducible promoter. Such constructs are well known to those of skill in the art. The Th2 immunostimulatory nucleic acid, however, does include plasmids and other vectors that are not expression vectors. That is, Th2 immunostimulatory nucleic acids include vectors that are not capable of expressing a peptide or peptide fragment. Th2 immunostimulatory nucleic acids, however, include plasmids and other vectors which cannot express a peptide or peptide fragment, i.e. plasmids which are partially or completely methylated of plasmids that are missing or have defective gene expression sequences or genes etc. In other embodiments, the Th2 immunostimulatory nucleic acids specifically exclude all vectors whether they are expression vectors or not.

[0067] In some embodiments the Th2 immunostimulatory nucleic acid is an oligonucleotide in the range of between 6 and 100 and more preferably between 6 and 50 nucleotides in size, and even more preferably 15-50 nucleotides in size. Alternatively, the Th2 immunostimulatory nucleic acid can be larger than 100 nucleotides in length.

[0068] The Th2 immunostimulatory nucleic acids may be a stabilized nucleic acid molecule. A "stabilized nucleic acid molecule" shall mean a nucleic acid molecule that is relatively resistant to in vivo degradation (e.g. via an exo- or endo-nuclease). Stabilization can be a function of length or secondary structure. Th2 immunostimulatory nucleic acids that are tens to hundreds of kbs long are relatively resistant to in vivo degradation. For shorter Th2 immunostimulatory nucleic acids, secondary structure can stabilize and increase their effect. For example, if the 3' end of an oligonucleotide has self-complementarity to an upstream region, so that it can fold back and form a sort of stem loop structure, then the oligonucleotide becomes stabilized and therefore exhibits more activity.

[0069] Some stabilized nucleic acids of the instant invention have a modified backbone. Modification of the nucleic acid backbone with, for example, phosphorothioate linkages provides enhanced activity of the Th2 immunostimulatory nucleic acids, in some aspects of the invention, when administered in vivo, and protects the nucleic acid from degradation by intracellular exo- and endo-nucleases. In other aspects, the backbone of the Th2 immunostimulatory is less important, and a phosphodiester backbone Th2 immunostimulatory nucleic acid is as effective as a phosphorothioate backbone Th2 immunostimulatory nucleic acid. As an example, when administered mucosally or dermally according to some aspects of the invention, Th2 immunostimulatory nucleic acids comprising a phosphodiester backbone, are as effective as phosphorothioate backbone counter-parts, and have the additional characteristic of inducing less of a Th1 immune response in the process. Other modified oligonucleotides include phosphodiester modified oligonucleotides, combinations of phosphodiester and phosphorothioate oligonucleotides, methylphosphonate, methylphosphorothioate, phosphorodithioate, and combinations thereof. Each of these combinations and their particular effects on immune cells is discussed, with respect to CpG oligonucleotides, in more detail in PCT Published Patent Application No. WO98/18810 claiming priority to U.S. Ser. No. 08/738,652, filed on Oct. 30, 1996, the entire contents of which are hereby incorporated by reference. It is believed that these modified oligonucleotides may show more stimulatory activity due to enhanced nuclease resistance, increased cellular uptake, increased protein binding, and/or altered intracellular localization.

[0070] Other stabilized oligonucleotides include: nonionic DNA analogs, such as alkyl- and aryl-phosphates (in which the charged phosphonate oxygen is replaced by an alkyl or aryl group), phosphodiester and alkylphosphotriesters, in which the charged oxygen moiety is alkylated. Oligonucleotides which contain diol, such as tetraethyleneglycol or hexaethyleneglycol, at either or both

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termini have also been shown to be substantially resistant to nuclease degradation.

[0071] In some instances stabilized nucleic acids are preferred because they are less susceptible to degradation. Nucleic acids, however, with other backbones may also be effective, although in cases where the backbone is nuclease sensitive, some form of formulation or delivery system may be preferred to protect the nucleic acids. Thus when a less stable nucleic acid is delivered to a subject, it is preferred that the nucleic acid be associated with a vehicle that delivers it directly into the cell. Such vehicles are known in the art and include, for example, liposomes and gene guns.

[0072] The Th2 immunostimulatory nucleic acid is administered to the subject with an antigen or in some cases the subject is exposed to the antigen to induce an antigen specific immune response. The antigen exposure may be active, e.g., the deliberate administration to a subject in need of such treatment, or passive. Passive exposure may occur prior to or following administration of the Th2 immune response. As an example, some of the prophylactic methods provided by the invention involve administration of Th2 immunostimulatory nucleic acids to subjects not yet exposed to an antigen but perhaps at risk of such exposure. An antigen specific immune response is an immune response characterized by the production of antibody which has specificity for an antigen. The antigen specific immune response may be a systemic or a mucosal immune response. As shown in the experiments described herein the Th2 immunostimulatory nucleic acids when administered in conjunction with the antigen produce IgG1 and in some cases IgG2a that are specific for the particular antigen. These antibodies are characteristic of a systemic immune response. The IgG2a is associated with a Th1 immune response and the IgG1 is associated with a Th2 immune response. Th2 immunostimulatory nucleic acids produce higher levels of IgG1 than IgG2a antibodies.

[0073] In addition to inducing systemic immune responses the Th2 immunostimulatory nucleic acids are also effective as mucosal adjuvants with many forms of antigen, such as those for which CT has been shown to be an effective adjuvant. This includes, but is not limited to, recombinant proteins, synthetic peptides, and attenuated or killed whole pathogens. Thus, in addition to the induction of Th2-biased systemic immune responses, the Th2 immunostimulatory nucleic acids can also augment antigen-specific mucosal immunity (i.e., secretory IgA), which helps protect against infection by preventing the entry of pathogens at mucosal surfaces. Owing to the existence of a common mucosal immune system, immunization with Th2 immunostimulatory nucleic acids at one mucosal surface can protect against infection by pathogens that enter via other mucosal routes (e.g., an oral vaccine could protect against a sexually transmitted disease or a respiratory infection). Thus the Th2 immunostimulatory nucleic acids are capable of inducing mucosal immunity in remote sites as well as local sites. A "remote site" as used herein is a mucosal tissue that is located in a different region of the body than the mucosal tissue to which the Th2 immunostimulatory nucleic acids has been administered. For instance if the Th2 immunostimulatory nucleic acids is administered intranasally, a remote site would be the mucosal lining of the gut.

[0074] The Th2 immunostimulatory nucleic acids are administered to subjects. A "subject" as used herein is a human or vertebrate animal including but not limited to a dog, cat, horse, cow, pig, sheep, goat, chicken, primate, e.g., monkey, fish (aquaculture species), e.g. salmon, rat, and mouse.

[0075] The subject is exposed to the antigen. As used herein, the term "exposed to" refers to either the active step of contacting the subject with an antigen or the passive exposure of the subject to the antigen. The term "administered" when used in conjunction with an antigen refers to the active step of bringing the subject in contact with the antigen. Methods for the active exposure, or administration, of an antigen to a subject are well-known in the art. In general, an antigen is administered directly to the subject by any means such as intravenous, intramuscular, oral, transdermal, mucosal, intranasal, intratracheal, or subcutaneous administration. The antigen can be administered systemically, mucosally, or locally.

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Methods for administering the antigen and the Th2 immunostimulatory nucleic acids are described in more detail below. A subject is passively exposed to an antigen if an antigen becomes available for exposure to the immune cells in the body. A subject may be passively exposed to an antigen, for instance, by entry of a foreign pathogen into the body or by the development of a tumor cell expressing a foreign antigen on its surface. When a subject is passively exposed to an antigen, in some embodiments the Th2 immunostimulatory nucleic acid is an oligonucleotide of 8-100 nucleotides in length and/or has a phosphate modified backbone.

[0076] The methods in which a subject is passively exposed to an antigen can be particularly dependent on timing of administration of the Th2 immunostimulatory nucleic acids. For instance, in a subject at risk of developing an infectious disease the subject may be administered the Th2 immunostimulatory nucleic acid on a regular basis when that risk is greatest, i.e., after exposure to an infectious agent. Additionally the Th2 immunostimulatory nucleic acids may be administered to travelers before they travel to foreign lands where they are at risk of exposure to infectious agents, especially Th1 mediated infectious agents. Likewise the Th2 immunostimulatory nucleic acids may be administered to soldiers or civilians at risk of exposure to biowarfare to induce an immune response to the antigen when and if the subject is exposed to it. It is particularly preferred when the infectious agent induces an extracellular infection such as extracellular parasites or obligate intracellular parasites.

[0077] An "antigen" as used herein is a molecule capable of provoking an immune response. Antigens include but are not limited to cells, cell extracts, proteins, polypeptides, peptides, polysaccharides, polysaccharide conjugates, peptide mimics of polysaccharides, lipids, glycolipids, carbohydrates, viruses and viral extracts and muticellular organisms such as parasites and allergens. The term antigen broadly includes any type of molecule which is recognized by a host immune system as being foreign. Antigens include but are not limited to microbial antigens. The term "antigen" does not encompass self-antigens, which are defined below. Preferably, the antigens of the invention are not conjugated to the Th2 immunostimulatory nucleic acids, and thus the antigen and nucleic acid may be administered on different schedules and by different routes from each other. In some important embodiments, the antigen is administered in low doses (i.e., doses that would not induce an immune response if administered alone). In other embodiments, the antigen is one known to be minimally immunogenic.

[0078] A "microbial antigen" as used herein is an antigen of a microorganism and includes but is not limited to infectious virus, infectious bacteria, infectious parasites, infectious yeast, and infectious fungi. Such antigens include the intact microorganism as well as natural isolates and fragments or derivatives thereof and also synthetic compounds which are identical to or similar to natural microorganism antigens and induce an immune response specific for that microorganism. A compound is similar to a natural microorganism antigen if it induces an immune response (humoral and/or cellular) to a natural microorganism antigen. Such antigens are used routinely in the art and are well known to those of ordinary skill in the art. Some microorganisms are associated with a Th1 -mediated disease and others are associated with a Th2-mediated disease. When the Th2 immunostimulatory nucleic acid is administered as an adjuvant in order to produce an antigen-specific immune response, it may be used against microorganisms that are associated with a Th1 or Th2 mediated disease, for the prevention and treatment of infection with those organisms. If the Th2 immunostimulatory nucleic acid is administered to a subject having an active bacterial or viral infection, the infection is preferably caused by a microbe not associated with a Th1 immunostimulatory nucleic acid.

[0079] An extracellular antigen as used herein is an antigen associated with an extracellular infection, preferably by a microbe that exists entirely extracellularly when in a host body and which also contains Th1 immunostimulatory nucleic acid. An example of an extracellular antigen is an antigen from a bacteria that contains Th1 immunostimulatory nucleic acids. Antigens that are not extracellular antigens, as described herein, are referred to as non-extracellular antigens. Non-extracellular antigens include, but

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are not limited to, tumor antigens or antigens derived from microbes that are not associated with a Th1 immunostimulatory nucleic acid. The methods of the invention generally intend to use in some aspects the Th2 immunostimulatory nucleic acids as adjuvants for extracellular antigens but preferably only when those extracellular antigens are not conjugated to the Th2 immunostimulatory antigens. Non-extracellular antigens are intended for use with the Th2 immunostimulatory nucleic acids of the invention, whether in a conjugated or non-conjugated form. In important embodiments, the non-extracellular antigens are not conjugated to the Th2 immunostimulatory nucleic acids.

[0080] Examples of virus that have been found in humans include but are not limited to: Retroviridae (e.g. human immunodeficiency viruses, such as HIV-1 (also referred to as HTLV-III, LAV or HTLV-III/LAV, or HIV-III; and other isolates, such as HIV-LP; Picornaviridae (e.g. polio viruses, hepatitis A virus; enteroviruses, human Coxsackie viruses, rhinoviruses, echoviruses); Calciviridae (e.g. strains that cause gastroenteritis); Togaviridae (e.g. equine encephalitis viruses, rubella viruses); Flaviridae (e.g. dengue viruses, encephalitis viruses, yellow fever viruses); Coronaviridae (e.g. coronaviruses); Rhabdoviridae (e.g. vesicular stomatitis viruses, rabies viruses); Filoviridae (e.g. ebola viruses); Paramyxoviridae (e.g. parainfluenza viruses, mumps virus, measles virus, respiratory syncytial virus); Orthomyxoviridae (e.g. influenza viruses); Bungaviridae (e.g. Hantaan viruses, bunga viruses, phleboviruses and Nairo viruses); Arena viridae (hemorrhagic fever viruses); Reoviridae (e.g. reoviruses, orbiviurses and rotaviruses); Birnaviridae; Hepadnaviridae (Hepatitis B virus); Parvovirida (parvoviruses); Papovaviridae (papilloma viruses, polyoma viruses); Adenoviridae (most adenoviruses); Herpesviridae (herpes simplex virus (HSV) 1 and 2, varicella zoster virus, cytomegalovirus (CMV), herpes virus; Poxviridae (variola viruses, vaccinia viruses, pox viruses); and Iridoviridae (e.g. African swine fever virus); and unclassified viruses (e.g. the etiological agents of Spongiform encephalopathies, the agent of delta hepatitis (thought to be a defective satellite of hepatitis B virus), the agents of non-A, non-B hepatitis (class 1=internally transmitted; class 2=parenterally transmitted (i.e. Hepatitis C); Norwalk and related viruses, and astroviruses).

[0081] Both gram negative and gram positive bacteria serve as antigens in vertebrate animals. Such gram positive bacteria include, but are not limited to Pasteurella species, Staphylococci species, and Streptococcus species. Gram negative bacteria include, but are not limited to, Escherichia coli, Pseudomonas species, and Salmonella species. Specific examples of infectious bacteria include but are not limited to: Helicobacter pyloris, Borelia burgdorferi, Legionella pneumophilia, Mycobacteria sps (e.g. M. tuberculosis, M. avium, M. intracellulare, M. kansaii, M. gordonae), Staphylococcus aureus, Neisseria gonorrhoeae, Neisseria meningitidis, Listeria monocytogenes, Streptococcus pyogenes (Group A Streptococcus), Streptococcus agalactiae (Group B Streptococcus), Streptococcus (viridans group), Streptococcus faecalis, Streptococcus bovis, Streptococcus (anaerobic sps.), Streptococcus pneumoniae, pathogenic Campylobacter sp., Enterococcus sp., Haemophilus influenzae, Bacillus antracis, corynebacterium diphtheriae, corynebacterium sp., Erysipelothrix rhusiopathiae, Clostridium perfringers, Clostridium tetani, Enterobacter aerogenes, Klebsiella pneumoniae, Pasturella multocida, Bacteroides sp., Fusobacterium nucleatum, Streptobacillus moniliformis, Treponema pallidium, Treponema pertenue, Leptospira, Rickettsia, and Actinomyces israelli.

[0082] Examples of fungi include: Cryptococcus neoformans, Histoplasma capsulatum, Coccidioides immitis, Blastomyces dermatitidis, Chlamydia trachomatis, Candida albicans. Other infectious organisms (i.e., protists) include: Plasmodium such as Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale, and Plasmodium vivax and Toxoplasma gondii.

[0083] Parasites include but are not limited to blood-borne and/or tissues parasites such as Plasmodium spp., Babesia microti, Babesia divergens, Leishmania tropica, Leishmania spp., Leishmania braziliensis, Leishmania donovani, Trypanosoma gambiense and Trypanosoma rhodesiense (African sleeping sickness), Trypanosoma cruzi (Chagas' disease), and Toxoplasma gondii.

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[0084] Other medically relevant microorganisms have been described extensively in the literature, e.g., see C.G.A Thomas, Medical Microbiology, Bailliere Tindall, Great Britain 1983, the entire contents of which is hereby incorporated by reference.

[0085] Although many of the microbial antigens described above relate to human disorders, the invention is also useful for treating other non-human vertebrates. Non-human vertebrates are also capable of developing infections which can be prevented or treated with the Th2 immunostimulatory nucleic acids disclosed herein. For instance, in addition to the treatment of infectious human diseases, the methods of the invention are useful for treating infections of animals.

[0086] As used herein, the term "treat", "treated", or "treating" when used with respect to an infectious disease refers to a prophylactic treatment which increases the resistance of a subject (a subject at risk of infection) to infection with a pathogen or, in other words, decreases the likelihood that the subject will become infected with the pathogen as well as a treatment after the subject (a subject who has been infected) has become infected in order to fight the infection, e.g., reduce or eliminate the infection or prevent it from becoming worse.

[0087] Many vaccines for the treatment of non-human vertebrates are disclosed in Bennett, K. Compendium of Veterinary Products, 3rd ed. North American Compendiums, Inc., 1995. As discussed above, antigens include infectious microbes such as virus, bacteria, parasites, and fungi and fragments thereof, derived from natural sources or synthetically. Infectious virus of both human and non-human vertebrates, include retroviruses, RNA viruses and DNA viruses. This group of retroviruses includes both simple retroviruses and complex retroviruses. The simple retroviruses include the subgroups of B-type retroviruses, C-type retroviruses and D-type retroviruses. An example of a B-type retrovirus is mouse mammary tumor virus (MMTV). The C-type retroviruses include subgroups C-type group A (including Rous sarcoma virus (RSV), avian leukemia virus (ALV), and avian myeloblastosis virus (AMV)) and C-type group B (including murine leukemia virus (MLV), feline leukemia virus (FeLV), murine sarcoma virus (MSV), gibbon ape leukemia virus (GALV), spleen necrosis virus (SNV), reticuloendotheliosis virus (RV) and simian sarcoma virus (SSV)). The D-type retroviruses include Mason-Pfizer monkey virus (MPMV) and simian retrovirus type 1 (SRV-1). The complex retroviruses include the subgroups of lentiviruses, T-cell leukemia viruses and the foamy viruses. Lentiviruses include HIV-1, but also include HIV-2, SIV, Visna virus, feline immunodeficiency virus (FIV), and equine infectious anemia virus (EIAV). The T-cell leukemia viruses include HTLV-1, HTLV-II, simian T-cell leukemia virus (STLV), and bovine leukemia virus (BLV). The foamy viruses include human foamy virus (HFV), simian foamy virus (SFV) and bovine foamy virus (BFV).

[0088] Examples of other RNA viruses that are antigens in vertebrate animals include, but are not limited to, the following: members of the family Reoviridae, including the genus Orthoreovirus (multiple serotypes of both mammalian and avian retroviruses), the genus Orbivirus (Bluetongue virus, Eugenangee virus, Kemerovo virus, African horse sickness virus, and Colorado Tick Fever virus), the genus Rotavirus (human rotavirus, Nebraska calf diarrhea virus, murine rotavirus, simian rotavirus, bovine or ovine rotavirus, avian rotavirus); the family Picornaviridae, including the genus Enterovirus (poliovirus, Coxsackie virus A and B, enteric cytopathic human orphan (ECHO) viruses, hepatitis A virus, Simian enteroviruses, Murine encephalomyelitis (ME) viruses, Poliovirus muris, Bovine enteroviruses, Porcine enteroviruses, the genus Cardiovirus (Encephalomyocarditis virus (EMC), Mengovirus), the genus Rhinovirus (Human rhinoviruses including at least 113 subtypes; other rhinoviruses), the genus Apthovirus (Foot and Mouth disease (FMDV); the family Calciviridae, including Vesicular exanthema of swine virus, San Miguel sea lion virus, Feline picornavirus and Norwalk virus; the family Togaviridae, including the genus Alphavirus (Eastern equine encephalitis virus, Semliki forest virus, Sindbis virus, Chikungunya virus, O'Nyong-Nyong virus, Ross river virus,

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Venezuelan equine encephalitis virus, Western equine encephalitis virus), the genus Flavirius (Mosquito borne yellow fever virus, Dengue virus, Japanese encephalitis virus, St. Louis encephalitis virus, Murray Valley encephalitis virus, West Nile virus, Kunjin virus, Central European tick borne virus, Far Eastern tick borne virus, Kyasanur forest virus, Louping III virus, Powassan virus, Omsk hemorrhagic fever virus), the genus Rubivirus (Rubella virus), the genus Pestivirus (Mucosal disease virus, Hog cholera virus, Border disease virus); the family Bunyaviridae, including the genus Bunyvirus (Bunyamwera and related viruses, California encephalitis group viruses), the genus Phlebovirus (Sandfly fever Sicilian virus, Rift Valley fever virus), the genus Nairovirus (Crimean-Congo hemorrhagic fever virus, Nairobi sheep disease virus), and the genus Uukuvirus (Uukuniemi and related viruses); the family Orthomyxoviridae, including the genus Influenza virus (Influenza virus type A, many human subtypes); Swine influenza virus, and Avian and Equine Influenza viruses; influenza type B (many human subtypes), and influenza type C (possible separate genus); the family paramyxoviridae, including the genus Paramyxovirus (Parainfluenza virus type 1, Sendai virus, Hemadsorption virus, Parainfluenza viruses types 2 to 5, Newcastle Disease Virus, Mumps virus), the genus Morbillivirus (Measles virus, subacute sclerosing panencephalitis virus, distemper virus, Rinderpest virus), the genus Pneumovirus (respiratory syncytial virus (RSV), Bovine respiratory syncytial virus and Pneumonia virus of mice); forest virus, Sindbis virus, Chikungunya virus, O'Nyong-Nyong virus, Ross river virus, Venezuelan equine encephalitis virus, Western equine encephalitis virus), the genus Flavirius (Mosquito borne yellow fever virus, Dengue virus, Japanese encephalitis virus, St. Louis encephalitis virus, Murray Valley encephalitis virus, West Nile virus, Kunjin virus, Central European tick borne virus, Far Eastern tick borne virus, Kyasanur forest virus, Louping III virus, Powassan virus, Omsk hemorrhagic fever virus), the genus Rubivirus (Rubella virus), the genus Pestivirus (Mucosal disease virus, Hog cholera virus, Border disease virus); the family Bunyaviridae, including the genus Bunyvirus (Bunyamwera and related viruses, California encephalitis group viruses), the genus Phlebovirus (Sandfly fever Sicilian virus, Rift Valley fever virus), the genus Nairovirus (Crimean-Congo hemorrhagic fever virus, Nairobi sheep disease virus), and the genus Uukuvirus (Uukuniemi and related viruses); the family Orthomyxoviridae, including the genus Influenza virus (Influenza virus type A, many human subtypes); Swine influenza virus, and Avian and Equine Influenza viruses; influenza type B (many human subtypes), and influenza type C (possible separate genus); the family paramyxoviridae, including the genus Paramyxovirus (Parainfluenza virus type 1, Sendai virus, Hemadsorption virus, Parainfluenza viruses types 2 to 5, Newcastle Disease Virus, Mumps virus), the genus Morbillivirus (Measles virus, subacute sclerosing panencephalitis virus, distemper virus, Rinderpest virus), the genus Pneumovirus (respiratory syncytial virus (RSV), Bovine respiratory syncytial virus and Pneumonia virus of mice); the family Rhabdoviridae, including the genus Vesiculovirus (VSV), Chandipura virus, Flanders-Hart Park virus), the genus Lyssavirus (Rabies virus), fish Rhabdoviruses, and two probable Rhabdoviruses (Marburg virus and Ebola virus); the family Arenaviridae, including Lymphocytic choriomeningitis virus (LCM), Tacaribe virus complex, and Lassa virus; the family Coronoaviridae, including Infectious Bronchitis Virus (IBV), Mouse Hepatitis virus, Human enteric corona virus, and Feline infectious peritonitis (Feline coronavirus).

[0089] Illustrative DNA viruses that are antigens in vertebrate animals include, but are not limited to: the family Poxviridae, including the genus Orthopoxvirus (Variola major, Variola minor, Monkey pox Vaccinia, Cowpox, Buffalopox, Rabbitpox, Ectromelia), the genus Leporipoxvirus (Myxoma, Fibroma), the genus Avipoxvirus (Fowlpox, other avian poxvirus), the genus Capripoxvirus (sheeppox, goatpox), the genus Suipoxvirus (Swinepox), the genus Parapoxvirus (contagious postular dermatitis virus, pseudocowpox, bovine papular stomatitis virus); the family Iridoviridae (African swine fever virus, Frog viruses 2 and 3, Lymphocystis virus of fish); the family Herpesviridae, including the alpha-Herpesviruses (Herpes Simplex Types 1 and 2, Varicella-Zoster, Equine abortion virus, Equine herpes virus 2 and 3, pseudorabies virus, infectious bovine keratoconjunctivitis virus, infectious bovine rhinotracheitis virus, feline rhinotracheitis virus, infectious laryngotracheitis virus) the Beta-herpesviruses (Human cytomegalovirus and cytomegaloviruses of swine, monkeys and rodents); the

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gamma-herpesviruses (Epstein-Barr virus (EBV), Marek's disease virus, Herpes saimiri, Herpesvirus ateles, Herpesvirus sylvilagus, guinea pig herpes virus, Lucke tumor virus); the family Adenoviridae, including the genus Mastadenovirus (Human subgroups A,B,C,D,E and ungrouped; simian adenoviruses (at least 23 serotypes), infectious canine hepatitis, and adenoviruses of cattle, pigs, sheep, frogs and many other species, the genus Aviadenovirus (Avian adenoviruses); and non-cultivatable adenoviruses; the family Papoviridae, including the genus Papillomavirus (Human papilloma viruses, bovine papilloma viruses, Shope rabbit papilloma virus, and various pathogenic papilloma viruses of other species), the genus Polyomavirus (polyomavirus, Simian vacuolating agent (SV-40), Rabbit vacuolating agent (RKV), K virus, BK virus, JC virus, and other primate polyoma viruses such as Lymphotrophic papilloma virus); the family Parvoviridae including the genus Adeno-associated viruses, the genus Parvovirus (Feline panleukopenia virus, bovine parvovirus, canine parvovirus, Aleutian mink disease virus, etc). Finally, DNA viruses may include viruses which do not fit into the above families such as Kuru and Creutzfeldt-Jacob disease viruses and chronic infectious neuropathic agents (CHINA virus).

[0090] Each of the foregoing lists is illustrative, and is not intended to be limiting.

[0091] In addition to the use of the Th2 immunostimulatory nucleic acids to induce an antigen specific immune response in humans, the methods of the preferred embodiments are particularly well suited for treatment of non-human vertebrates. Non-human vertebrates which exist in close quarters and which are allowed to intermingle as in the case of zoo, farm and research animals are also embraced as subjects for the methods of the invention. Zoo animals such as the felid species including for example lions, tigers, leopards, cheetahs, and cougars; elephants, giraffes, bears, deer, wolves, yaks, non-human primates, seals, dolphins and whales; and research animals such as mice, rats, hamsters and gerbils are all potential subjects for the methods of the invention.

[0092] Birds such as hens, chickens, turkeys, ducks, geese, quail, and pheasant are prime targets for many types of infections. Hatching birds are exposed to pathogenic microorganisms shortly after birth. Although these birds are initially protected against pathogens by maternal derived antibodies, this protection is only temporary, and the bird's own immature immune system must begin to protect the bird against the pathogens. It is often desirable to prevent infection in young birds when they are most susceptible. It is also desirable to prevent against infection in older birds, especially when the birds are housed in closed quarters, leading to the rapid spread of disease. Thus, it is desirable to administer the Th2 immunostimulatory nucleic acid to birds to enhance an antigen-specific immune response when antigen is present.

[0093] An example of a common infection in chickens is chicken infectious anemia virus (CIAV). CIAV was first isolated in Japan in 1979 during an investigation of a Marek's disease vaccination break (Yuasa et al., 1979, Avian Dis. 23:366-385). Since that time, CIAV has been detected in commercial poultry in all major poultry producing countries (van Bulow et al., 1991, pp. 690-699) in Diseases of Poultry, 9th edition, Iowa State University Press).

[0094] CIAV infection results in a clinical disease, characterized by anemia, hemorrhage and immunosuppression, in young susceptible chickens. Atrophy of the thymus and of the bone marrow and consistent lesions of CIAV-infected chickens are also characteristic of CIAV infection. Lymphocyte depletion in the thymus, and occasionally in the bursa of Fabricius, results in immunosuppression and increased susceptibility to secondary viral, bacterial, or fungal infections which then complicate the course of the disease. The immunosuppression may cause aggravated disease after infection with one or more of Marek's disease virus (MDV), infectious bursal disease virus, reticuloendotheliosis virus, adenovirus, or reovirus. It has been reported that pathogenesis of MDV is enhanced by CIAV (DeBoer et al., 1989, p. 28 In Proceedings of the 38th Western Poultry Diseases Conference, Tempe, Ariz.). Further, it has been reported that CIAV aggravates the signs of infectious bursal disease (Rosenberger et

al., 1989, Avian Dis. 33:707-713). Chickens develop an age resistance to experimentally induced disease due to CAA. This is essentially complete by the age of 2 weeks, but older birds are still susceptible to infection (Yuasa, N. et al., 1979 supra; Yuasa, N. et al., Arian Diseases 24, 202-209, 1980). However, if chickens are dually infected with CAA and an immunosuppressive agent (IBDV, MDV etc.) age resistance against the disease is delayed (Yuasa, N. et al., 1979 and 1980 supra; Bulow von V. et al., J. Veterinary Medicine 33, 93-116, 1986). Characteristics of CIAV that may potentiate disease transmission include high resistance to environmental inactivation and some common disinfectants. The economic impact of CIAV infection on the poultry industry is clear from the fact that 10% to 30% of infected birds in disease outbreaks die.

[0095] Vaccination of birds, like other vertebrate animals can be performed at any age. Normally, vaccinations are performed at up to 12 weeks of age for a live microorganism and between 14-18 weeks for an inactivated microorganism or other type of vaccine. For in ovo vaccination, vaccination can be performed in the last quarter of embryo development. The vaccine may be administered subcutaneously, by spray, orally, intraocularly, intratracheally, nasally, or by other mucosal delivery methods described herein. Thus, the Th2 immunostimulatory nucleic acid can be administered to birds and other non-human vertebrates using routine vaccination schedules and the antigen is administered after an appropriate time period as described herein.

[0096] Cattle and livestock are also susceptible to infection. Disease which affect these animals can produce severe economic losses, especially amongst cattle. The methods of the invention can be used to protect against infection in livestock, such as cows, horses, pigs, sheep, and goats.

[0097] Cows can be infected by bovine viruses. Bovine viral diarrhea virus (BVDV) is a small enveloped positive-stranded RNA virus and is classified, along with hog cholera virus (HOCV) and sheep border disease virus (BDV), in the pestivirus genus. Although, Pestiviruses were previously classified in the Togaviridae family, some studies have suggested their reclassification within the Flaviviridae family along with the flavivirus and hepatitis C virus (HCV) groups (Francki, et al., 1991).

[0098] BVDV, which is an important pathogen of cattle can be distinguished, based on cell culture analysis, into cytopathogenic (CP) and noncytopathogenic (NCP) biotypes. The NCP biotype is more widespread although both biotypes can be found in cattle. If a pregnant cow becomes infected with an NCP strain, the cow can give birth to a persistently infected and specifically immunotolerant calf that will spread virus during its lifetime. The persistently infected cattle can succumb to mucosal disease and both biotypes can then be isolated from the animal. Clinical manifestations can include abortion, teratogenesis, and respiratory problems, mucosal disease and mild diarrhea. In addition, severe thrombocytopenia, associated with herd epidemics, that may result in the death of the animal has been described and strains associated with this disease seem more virulent than the classical BVDVs.

[0099] Equine herpesviruses (EHV) comprise a group of antigenically distinct biological agents which cause a variety of infections in horses ranging from subclinical to fatal disease. These include Equine herpesvirus-1 (EHV-1), a ubiquitous pathogen in horses. EHV-1 is associated with epidemics of abortion, respiratory tract disease, and central nervous system disorders. Primary infection of upper respiratory tract of young horses results in a febrile illness which lasts for 8 to 10 days. Immunologically experienced mares may be reinfected via the respiratory tract without disease becoming apparent, so that abortion usually occurs without warning. The neurological syndrome is associated with respiratory disease or abortion and can affect animals of either sex at any age, leading to incoordination, weakness and posterior paralysis (Telford, E. A. R. et al., Virology 189, 304-316, 1992). Other EHV's include EHV-2, or equine cytomegalovirus, EHV-3, equine coital exanthema virus, and EHV-4, previously classified as EHV-1 subtype 2.

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[0100] Sheep and goats can be infected by a variety of dangerous microorganisms including visna-maedi.

[0101] Primates such as monkeys, apes and macaques can be infected by simian immunodeficiency virus. Inactivated cell-virus and cell-free whole simian immunodeficiency vaccines have been reported to afford protection in macaques (Stott et al. (1990) Lancet 36:1538-1541; Desrosiers et al. PNAS USA (1989) 86:6353-6357; Murphey-Corb et al. (1989) Science 246:1293-1297; and Carlson et al. (1990) AIDS Res. Human Retroviruses 6:1239-1246). A recombinant HIV gp120 vaccine has been reported to afford protection in chimpanzees (Berman et al. (1990) Nature 345:622-625).

[0102] Cats, both domestic and wild, are susceptible to infection with a variety of microorganisms. For instance, feline infectious peritonitis is a disease which occurs in both domestic and wild cats, such as lions, leopards, cheetahs, and jaguars. When it is desirable to prevent infection with this and other types of pathogenic organisms in cats, the methods of the invention can be used to vaccinate cats to protect them against infection.

[0103] Domestic cats may become infected with several retroviruses, including but not limited to feline leukemia virus (FeLV), feline sarcoma virus (FeSV), endogenous type C oncornavirus (RD-114), and feline syncytia-forming virus (FeSFV). Of these, FeLV is the most significant pathogen, causing diverse symptoms, including lymphoreticular and myeloid neoplasms, anemias, immune mediated disorders, and an immunodeficiency syndrome which is similar to human acquired immune deficiency syndrome (AIDS). Recently, a particular replication-defective FeLV mutant, designated FeLV-AIDS, has been more particularly associated with immunosuppressive properties.

[0104] The discovery of feline T-lymphotropic lentivirus (also referred to as feline immunodeficiency) was first reported in Pedersen et al. (1987) Science 235:790-793. Characteristics of FIV have been reported in Yamamoto et al. (1988) Leukemia, December Supplement 2:204S-215S; Yamamoto et al. (1988) Am. J. Vet. Res. 49:1246-1258; and Ackley et al. (1990) J. Virol. 64:5652-5655. Cloning and sequence analysis of FIV have been reported in Olmsted et al. (1989) Proc. Natl. Acad. Sci. USA 86:2448-2452 and 86:4355-4360.

[0105] Feline infectious peritonitis (FIP) is a sporadic disease occurring unpredictably in domestic and wild Felidae. While FIP is primarily a disease of domestic cats, it has been diagnosed in lions, mountain lions, leopards, cheetahs, and the jaguar. Smaller wild cats that have been afflicted with FIP include the lynx and caracal, sand cat, and pallas cat. In domestic cats, the disease occurs predominantly in young animals, although cats of all ages are susceptible. A peak incidence occurs between 6 and 12 months of age. A decline in incidence is noted from 5 to 13 years of age, followed by an increased incidence in cats 14 to 15 years old.

[0106] Viral, bacterial, and parasitic diseases in fin-fish, shellfish or other aquatic life forms pose a serious problem for the aquaculture industry. Owing to the high density of animals in the hatchery tanks or enclosed marine farming areas, infectious diseases may eradicate a large proportion of the stock in, for example, a fin-fish, shellfish, or other aquatic life forms facility. Prevention of disease is a more desired remedy to these threats to fish than intervention once the disease is in progress. Vaccination of fish is the only preventative method which may offer long-term protection through immunity. Nucleic acid based vaccinations are described in U.S. Pat. No. 5,780,448 issued to Davis.

[0107] The fish immune system has many features similar to the mammalian immune system, such as the presence of B cells, T cells, lymphokines, complement, and immunoglobulins. Fish have lymphocyte subclasses with roles that appear similar in many respects to those of the B and T cells of mammals. Vaccines can be administered by immersion or orally.

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[0108] Aquaculture species include but are not limited to fin-fish, shellfish, and other aquatic animals. Fin-fish include all vertebrate fish, which may be bony or cartilaginous fish, such as, for example, salmonids, carp, catfish, yellowtail, seabream, and seabass. Salmonids are a family of fin-fish which include trout (including rainbow trout), salmon, and Arctic char. Examples of shellfish include, but are not limited to, clams, lobster, shrimp, crab, and oysters. Other cultured aquatic animals include, but are not limited to eels, squid, and octopi.

[0109] Polypeptides of viral aquaculture pathogens include but are not limited to glycoprotein (G) or nucleoprotein (N) of viral hemorrhagic septicemia virus (VHSV); G or N proteins of infectious hematopoietic necrosis virus (IHNV); VP1, VP2, VP3 or N structural proteins of infectious pancreatic necrosis virus (IPNV); G protein of spring viremia of carp (SVC); and a membrane-associated protein, tegumin or capsid protein or glycoprotein of channel catfish virus (CCV).

[0110] Polypeptides of bacterial pathogens include but are not limited to an iron-regulated outer membrane protein, (IROMP), an outer membrane protein (OMP), and an A-protein of Aeromonis salmonicida which causes furunculosis, p57 protein of Renibacterium salmoninarum which causes bacterial kidney disease (BKD), major surface associated antigen (msa), a surface expressed cytotoxin (mpr), a surface expressed hemolysin (ish), and a flagellar antigen of Yersiniosis; an extracellular protein (ECP), an iron-regulated outer membrane protein (IROMP), and a structural protein of Pasteurellosis; an OMP and a flagellar protein of Vibrosis anguillarum and V. ordalii; a flagellar protein, an OMP protein, aroA, and purA of Edwardsiellosis ictaluri and E. tarda; and surface antigen of Ichthyophthirius; and a structural and regulatory protein of Cytophaga columnari; and a structural and regulatory protein of Rickettsia.

[0111] Polypeptides of a parasitic pathogen include but are not limited to the surface antigens of Ichthyophthirius. Typical parasites infecting horses are Gasterophilus spp.; Eimeria leuckarti, Giardia spp.; Tritrichomonas equi; Babesia spp. (RBC's), Theileria equi; Trypanosoma spp.; Klossiella equi; Sarcocystis spp.

[0112] Typical parasites infecting swine include Eimeria bebliecki, Eimeria scabra, Isospora suis, Giardia spp.; Balantidium coli, Entamoeba histolytica; Toxoplasma gondii and Sarcocystis spp., and Trichinella spiralis.

[0113] The major parasites of dairy and beef cattle include Eimeria spp., Cryptosporidium sp., Giardia sp., Toxoplasma gondii; Babesia bovis (RBC), Babesia bigemina (RBC), Trypanosoma spp. (plasma), Theileria spp. (RBC); Theileria parva (lymphocytes); Tritrichomonas foetus; and Sarcocystis spp.

[0114] The major parasites of raptors include Trichomonas gallinae; Coccidia (Eimeria spp.); Plasmodium relictum, Leucocytozoon danilewskyi (owls), Haemoproteus spp., Trypanosoma spp.; Histomonas; Cryptosporidium meleagridis, Cryptosporidium baileyi, Giardia, Eimeria; Toxoplasma.

[0115] Typical parasites infecting sheep and goats include Eimeria spp., Cryptosporidium sp., Giardia sp.; Toxoplasma gondii; Babesia spp. (RBC), Trypanosoma spp. (plasma), Theileria spp. (RBC); and Sarcocystis spp.

[0116] Typical parasitic infections in poultry include coccidiosis caused by Eimeria acervulina, E. necatrix, E. tenella, Isospora spp. and Eimeria truncata; histomoniasis, caused by Histomonas meleagridis and Histomonas gallinarum; trichomoniasis caused by Trichomonas gallinae; and hexamitiasis caused by Hexamita meleagridis. Poultry can also be infected Emeria maxima, Emeria meleagridis, Eimeria adenoeides, Eimeria meleagrimitis, Cryptosporidium, Eimeria brunetti, Emeria

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adenoeides, Leucocytozoon spp., Plasmodium spp., Hemoproteus meleagridis, Toxoplasma gondii and Sarcocystis.

[0117] Parasitic infections also pose serious problems in laboratory research settings involving animal colonies. Some examples of laboratory animals intended to be treated, or in which parasite infection is sought to be prevented, by the methods of the invention include mice, rats, rabbits, guinea pigs, nonhuman primates, as well as the aforementioned swine and sheep.

[0118] Typical parasites in mice include Leishmania spp., Plasmodium berghei, Plasmodium yoelii, Giardia muris, Hexamita muris; Toxoplasma gondii; Trypanosoma duttoni (plasma); Kiossiella muris; Sarcocystis spp. Typical parasites in rats include Giardia muris, Hexamita muris; Toxoplasma gondii; Trypanosoma lewisi (plasma); Trichinella spiralis; Sarcocystis spp. Typical parasites in rabbits include Eimeria sp.; Toxoplasma gondii; Nosema cuniculi; Eimeria stiedae, Sarcocystis spp. Typical parasites of the hamster include Trichomonas spp.; Toxoplasma gondii; Trichinella spiralis; Sarcocystis spp. Typical parasites in the guinea pig include Balantidium caviae; Toxoplasma gondii; Klossiella caviae; Sarcocystis spp.

[0119] The methods of the invention can also be applied to the treatment and/or prevention of parasitic infection in dogs, cats, birds, fish and ferrets. Typical parasites of birds include Trichomonas gallinae; Eimeria spp., Isospora spp., Giardia; Cryptosporidium; Sarcocystis spp., Toxoplasma gondii, Haemoproteus/Parahaemoproteus, Plasmodium spp., LeucocytozoonlAkiba, Atoxoplasma, Trypanosoma spp. Typical parasites infecting dogs include Trichinella spiralis; Isopora spp., Sarcocystis spp., Cryptosporidium spp., Hammondia spp., Giardia duodenalis (canis); Balantidium coli, Entamoeba histolytica; Hepatozoon canis; Toxoplasma gondii, Trypanosoma cruzi; Babesia canis, Leishmania amastigotes; Neospora caninum.

[0120] Typical parasites infecting feline species include Isospora spp., Toxoplasma gondii, Sarcocystis spp., Hammondia hammondi, Besnoitia spp., Giardia spp.; Entamoeba histolytica; Hepatozoon canis, Cytauxzoon sp., Cytauxzoon sp., Cytauxzoon sp. (red cells, RE cells).

[0121] Typical parasites infecting fish include Hexamita spp., Eimeria spp.; Cryptobia spp., Nosema spp., Myxosoma spp., Chilodonella spp., Trichodina spp.; Plistophora spp., Myxosoma Henneguya; Costia spp., Ichthyophithirius spp., and Oodinium spp.

[0122] Typical parasites of wild mammals include Giardia spp. (carnivores, herbivores), Isospora spp. (carnivores), Eimeria spp. (carnivores, herbivores); Theileria spp. (herbivores), Babesia spp. (carnivores, herbivores), Trypanosoma spp. (carnivores, herbivores); Schistosoma spp. (herbivores); Fasciola hepatica (herbivores), Fascioloides magna (herbivores), Fasciola gigantica (herbivores), Trichinella spiralis (carnivores, herbivores).

[0123] Parasitic infections in zoos can also pose serious problems. Typical parasites of the bovidae family (blesbok, antelope, banteng, eland, gaur, impala, klipspringer, kudu, gazelle) include Eimeria spp. Typical parasites in the pinnipedae family (seal, sea lion) include Eimeria phocae. Typical parasites in the camelidae family (camels, llamas) include Eimeria spp. Typical parasites of the giraffidae family (giraffes) include Eimeria spp. Typical parasites in the elephantidae family (African and Asian) include Fasciola spp. Typical parasites of lower primates (chimpanzees, orangutans, apes, baboons, macaques, monkeys) include Giardia sp.; Balantidium coli, Entamzoeba histolytica, Sarcocystis spp., Toxoplasma gondii; Plasmodim spp. (RBC), Babesia spp. (RBC), Trypanosoma spp. (plasma), Leishmania spp. (macrophages).

[0124] In addition to producing antigen-specific immune responses, the invention is also useful for

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inducing a Th2 immune response in a subject. When a subject is administered a Th2-immunostimulatory nucleic acid a Th2 immune response is produced. Thus, Th2 immunostimulatory nucleic acids can also be given on their own to establish a more Th2 environment or to treat Th1 -mediated disorders. Importantly, in some aspects, the Th1 mediated disorders are not those induced by the presence of Th1 immunostimulatory nucleic acids, especially those containing an unmethylated CpG dinucleotide, deriving from some bacterial and viral infections. Although Th1 mediated disorders display similar characteristics regardless of whether they are induced by the presence of microbial derived Th1 immunostimulatory nucleic acids or not, the invention intends to treat preferably only those of this latter category.

[0125] It was discovered according to the invention that Th2 immunostimulatory nucleic acids induced predominantly Th2-like responses (IgG1>>IgG2a), whereas CpG nucleic acids resulted in mixed Th1/Th2 or predominantly Th1-like responses. Th2 responses in some instances are also considered mixed immune response that are nonetheless biased towards a Th2 profile. Th2 responses are highly desirable for the prevention or treatment of a number of Th1-mediated diseases including: organ-specific autoimmune disorders, Crohn's disease, Helicobacter pylori-induced peptic ulcer, acute solid organ allograft rejection, and unexplained recurrent abortion. The only adjuvant currently licensed for use in humans in most countries of the world, including the USA, is aluminum hydroxide (alum) which, although having a Th2 immunostimulatory effect, is weak, is associated with undesirable local tissue reactions, and is generally considered unsuitable for mucosal delivery. CT, which also enhances Th2-like immune responses, can be given mucosally, however it is too toxic for use in humans. A mouse (.about.20 g body weight) can tolerate the toxic effects of up to 10 .mu.g of CT, however a dose as little as 1-5 .mu.g will cause severe diarrhea in a human (.about.70 kg body weight) (Jertborn et al., 1992). Animals receiving Th2 immunostimulatory nucleic acids showed no short-term signs of distress over those receiving antigen alone, and all recovered quickly with no apparent long-lasting effects even with doses of up to 500 .mu.g. This is the first report of mucosal application of Th2 immunostimulatory nucleic acids to augment immune responses and the Th2-bias of the responses induced by Th2 immunostimulatory nucleic acids is of great importance in the development of effective Th2 biased prophylactic or therapeutic strategies.

[0126] Thus a subject, according to the invention, is a subject in need of a particular treatment. For instance, a subject may be a subject as risk of developing a disease such as cancer or an infectious disease or a subject that actually has cancer or an infectious disease. These subjects are administered the Th2 immunostimulatory nucleic acid of the invention, possibly in conjunction with an antigen to produce an antigen specific immune response to treat the cancer or infectious disease, thus preventing it from developing or from progressing, or alone to induce an antigen non-specific immune response.

[0127] Other subjects according to the invention are those that have or are at risk of developing a Th1 mediated disease. A "Th1 mediated disease" as used herein refers to a disease that is associated with the development of a Th1 immune response. A "Th1 immune response" as used herein refers to the induction of at least one Th1-cytokine or a Th1-antibody. In preferred embodiments more than one Th1-cytokine or Th1-antibody is induced. Thus a Th1-mediated disease is a disease associated with the induction of a Th1 response and refers to the partial or complete induction of at least one Th1-cytokine or Th1-antibody or an increase in the levels of at least one Th1-cytokine or Th1-antibody. These disorders are known in the art and include for instance, but are not limited to, autoimmune especially organ-specific autoimmune disease, psoriasis, Th1 inflammatory disorders, infection with extracellular parasites (e.g., response to helminths), solid organ allograft rejection (e.g., acute kidney allograft rejection), symptoms associated with hepatitis B (HBV) infection (e.g., HBV acute phase or recovery phase), chronic hepatitis C (HCV) infection, insulin-dependent diabetes mellitus (IDDM), multiple sclerosis (MS), "silent thyroiditis", Crohn's disease, primary biliary cirrhosis, primary sclerosing cholangitis, sarcoidosis, atherosclerosis, acute graft versus host disease (GvHD), glomerulonephritis,

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anti-glomerular basement membrane disease, Wegener's granulomatosis, inflammatory myopathies, Sjogren's syndrome, Behget's syndrome, rheumatoid arthritis, Lyme arthritis, and unexplained recurrent abortion. Some Th1 mediated diseases and references where they are described are set forth below.

1 Crohn's disease/IBD Kakazu T et al., Type I T-helper cell predominance in granulomas of Crohnrs disease. Am J Gastroenterol 1999 Aug;94(8):2149-55; Monteleone G et al., Bioactive IL-18 expression is up-regulated in Crolin's disease. J Immunol 1999 Jul 1;163(1):143-7; Camoglio L et al., Altered expression of interferon-gamma and interleukin- 4 in inflammatory bowel disease. Inflamm Bowel Dis 1998 Nov;4(4):285-90; Plevy SE et al., A role for TNF-alpha and mucosal T helper-1 cytokines in the pathogenesis of Crohnts disease. J Immunol 1997 Dec 15;1 59(12):6276-82; Noguchi M et al., Enhanced interferon-gamma production and B7-2 expression in isolated intestinal mononuclear cells from patients with Crohnts disease. J Gastroenterol 1995 Nov;30 Suppl 8:52-5. H. pylori Hida N et al., Increased expression of IL-10 and IL-12 (p40) mRNA in Helicobacter pylori infected gastric mucosa: relation to bacterial cag status and peptic ulceration. J Clin Pathol 1999 Sep;52(9):658-64; Mattapallil JJ et al., A predominant Th1 type of immune response is induced early during acute Helicobacter pylori infection in rhesus macaques. Gastroenterology 2000 Feb;118(2):307-15. Autoimmune Okazaki K et al., Autoimmune-related pancreatitis is associated with pancreatitis autoantibodies and a Th1/Th2-type cellular immune response. Gastroenterology 2000 Mar;118(3):573-81. Chronic hepatitis C Bertoletti A et al., Different cytokine profiles of intraphepatic T cells in chronic hepatitis B and hepatitis C virus infections. Gastroenterology 1997 Jan;112(1):193-9; Quiroga JA et al., Induction of interleukin- 12 production in chronic hepatitis C virus infection correlates with the hepatocellular damage. J Infect Dis 1998 Jul;178(1):247-51. Behcet's Syndrome Sugi-Ikai N et al., Increased frequencies of interleukin-2- and interferon- gamma-producing T cells in patients with active Behcet's disease. Invest Ophthalmol Vis Sci 1998 May;39(6):996-1004. PBC Dienes HP et al., Bile duct epithelia as target cells in primary biliary cirrhosis and primary sclerosing cholangitis. Virchows Arch 1997 Aug;43 1(2): 119-24; Tjandra K et al., Progressive development of a Thi-type hepatic cytokine profile in rats with experimental cholangitis. Hepatology 2000 Feb;3 1(2):280-90; Harada K et al., In situ nucleic acid hybridization of cytokines in primary biliary cirrhosis: predominance of the Th1 subset. Hepatology 1997 Apr;25(4):79 1-6. PSC Dienes HP et al., Bile duct epithelia as target cells in primary biliary cirrhosis and primary sclerosing cholangitis. Virchows Arch 1997 Aug;43 1(2): 119-24; Tjandra K et al., Progressive development of a Th1-type hepatic cytokine profile in rats with experimental cholangitis. Hepatology 2000 Feb;31(2):280-90. Sarcoidosis Moller DR, Cells and cytokines involved in the pathogenesis of sarcoidosis. Sarcoidosis Vasc Difuse Lung Dis 1999 Mar;16(1):24-31; Moller DR et al., Enhanced expression of IL-12 associated with Th1 cytokine profiles in active pulmonary sarcoidosis. J Immunol 1996 Jun 15; 156(12):4952-60. Atherosclerosis Frostegard J et al., Cytokine expression in advanced human atherosclerotic plaques: dominance of pro-inflammatory (Th 1) and macrophage- stimulating cytokines. Atherosclerosis 1999 Jul; 145(1): 33-43. Acute GvHD Ochs LA et al., Cytokine expression in human cutaneous chronic graft- versus-host disease. Bone Marrow Transplant 1996 Jun;17(6):1085-92; Williamson B et al., Neutralizing IL-12 during induction of murine acute graft-versus-host disease polarizes the cytokine profile toward a Th2- type alloimmune response and confers long term protection from disease. J Immunol 1997 Aug 1;159(3): 1208-15. Glomerulonephritis Kitching AR et al., IFN-gamma mediates crescent formation and cell- mediated immune injury in murine glomerulonephritis. J Am Soc Nephrol 1999 Apr;10(4):752-9; Holdsworth SR et al., Th1 and Th2 T helper cell subsets affect patterns of injury and outcomes in glomerulonephritis. Kidney Int 1999 Apr;55(4):1198-216. Wegener's Gross WL et al., Pathogenesis of Wegener's granulomatosis. Ann Med granulomatosis Interne (Paris) 1998 Sep; 149(5):280-6. Anti-GBM disease Kalluri R et al., Susceptibility to anti-glomerular basement membrane disease and Goodpasture syndrome is linked to MHC class II genes and the emergence of T cell-mediated immunity in mice. J Clin Invest 1997 Nov 1;100(9):2263-75; Coelho SN et al., Immunologic determinants of susceptibility to experimental glomerulonepliritis: role of cellular immunity. Kidney Int 1997 Mar;51(3):646-52. Lepidi H et al., Local expression of cytokines in idiopathic inflammatory myopathies. Neuropathol Appl Neurobiol 1998 Feb;24(1): 73-9. Siogren's

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syndrome Kolkowski BC et al., Th1 predominance and perform expression in minor salivary glands from patients with primary Sjogren's syndrome. J Autoimmun 1999 Aug;13(1):155-62. Lyme arthritis Yin Z et al., T cell cytokine pattern in the joints of patients with Lyme arthritis and its regulation by cytokines and anticytokines. Arthritis Rheum 1997 Jan;40(1):69-79. Rheumatoid arthritis Kusaba M et al., Analysis of type 1 and type 2 T cells in synovial fluid and peripheral blood of patients with rheumatoid arthritis. J Rheumatol 1998 Aug;25(8):1466-71.

[0128] As described above, when Th2 immunostimulatory nucleic acids are administered parenterally with antigen to produce an antigen-specific immune response, higher doses of the Th2 immunostimulatory nucleic acid are required than are required for mucosal administration. When the Th2 immunostimulatory nucleic acid is administered in combination with a therapeutic agent, higher doses are not required. Additionally, when the Th2 immunostimulatory nucleic acid is administered in order to induce a Th2 immune response or ADCC, higher doses are not required.

[0129] Autoimmune disease is a class of diseases in which an subject's own antibodies react with host tissue or in which immune effector T cells are autoreactive to endogenous self peptides and cause destruction of tissue. Thus an immune response is mounted against a subject's own antigens, referred to as self antigens. Autoimmune diseases include but are not limited to rheumatoid arthritis, Crohn's disease, multiple sclerosis, systemic lupus erythematosus (SLE), autoimmune encephalomyelitis, myasthenia gravis (MG), Hashimoto's thyroiditis, Goodpasture's syndrome, pemphigus (e.g., pemphigus vulgaris), Grave's disease, autoimmune hemolytic anemia, autoimmune thrombocytopenic purpura, scleroderma with anti-collagen antibodies, mixed connective tissue disease, polymyositis, pernicious anemia, idiopathic Addison's disease, autoimmune-associated infertility, glomerulonephritis (e.g., crescentic glomerulonephritis, proliferative glomerulonephritis), bullous pemphigoid, Sjogren's syndrome, insulin resistance, and autoimmune diabetes mellitus.

[0130] A "self-antigen" as used herein refers to an antigen of a normal host tissue. Normal host tissue does not include cancer cells. Thus an immune response mounted against a self-antigen, in the context of an autoimmune disease, is an undesirable immune response and contributes to destruction and damage of normal tissue, whereas an immune response mounted against a cancer antigen is a desirable immune response and contributes to the destruction of the tumor or cancer. Thus, in some aspects of the invention aimed at treating autoimmune disorders it is not recommended that the Th2 immunostimulatory nucleic acids be administered with self antigens, particularly those that are the targets of the autoimmune disorder.

[0131] A number of animal studies have demonstrated that mucosal administration of low doses of antigen can result in a state of immune hyporesponsiveness or "tolerance." The active mechanism appears to be a cytokine-mediated immune deviation away from a Th1 towards a predominantly Th2 and Th3 (i.e., TGF-.beta. dominated) response. The active suppression with low dose antigen delivery can also suppress an unrelated immune response (bystander suppression) which is of considerable interest in the therapy of autoimmune diseases, for example, rheumatoid arthritis and SLE. Bystander suppression involves the secretion of Th1 -counter-regulatory, suppressor cytokines in the local environment where proinflammatory and Th1 cytokines are released in either an antigen-specific or antigen-nonspecific manner. "Tolerance" as used herein is used to refer to this phenomenon. Indeed, oral tolerance has been effective in the treatment of a number of autoimmune diseases in animals including: experimental autoimmune encephalomyelitis (EAE) (Karpus et al., 1998, Rott et al., 1993, Chen et al., 1994), experimental autoimmune myasthenia gravis (Im et al., 1999, Ma et al., 1996), collagen-induced arthritis (CIA) (Nagler-Anderson et al., 1986), and insulin-dependent diabetes mellitus (Reddy et al., 2000, Ploix et al., 1998). In these models, the prevention and suppression of autoimmune disease is associated with a shift in antigen-specific humoral and cellular responses from a Th1 to Th2/Th3 response. Likewise, the Th2 immunostimulatory nucleic acids can also be used to promote Th2

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responses in the treatment of multiple sclerosis and other Th1 -associated inflammatory disorders. This could be accomplished by the use of Th2 immunostimulatory nucleic acids on its own, or in association with a self-antigen (e.g., collagen for treatment of rheumatoid arthritis, or SLE, nuclear and nucleolar antigens for scleroderma).

[0132] The methods of the invention are also useful for preventing or treating disease associated with extracellular parasitic infections. Most parasites are host-specific or have a limited host range, i.e., they are able to infect a single or at most a few species. For example, P. yoelii is able to infect only rodents while P. falciparum and P. malariae are able to infect humans. The parasitic infection to be targeted by the methods and compounds of the invention will depend upon the host species receiving the prophylactic treatment and the conditions to which that host will become exposed.

[0133] Parasites can be classified based on whether they are intracellular or extracellular. An "intracellular parasite" as used herein is a parasite whose entire life cycle is intracellular. Examples of human intracellular parasites include Leishmania spp., Plasmodium spp., Trypanosoma cruzi, Toxoplasma gondii, Babesia spp., and Trichinella spiralis. An "extracellular parasite" as used herein is a parasite whose entire life cycle is extracellular. Extracellular parasites capable of infecting humans include Entamoeba histolytica, Giardia lamblia, Enterocytozoon bieneusi, Naegleria and Acanthamoeba as well as most helminths. Yet another class of parasites is defined as being mainly extracellular but with an obligate intracellular existence at a critical stage in their life cycles. Such parasites are referred to herein as "obligate intracellular parasites". These parasites may exist most of their lives or only a small portion of their lives in an extracellular environment, but they all have at least one obligate intracellular stage in their life cycles. This latter category of parasites includes Trypanosoma rhodesiense and Trypanosoma gambiense, Isospora spp., Cryptosporidium spp, Eimeria spp., Neospora spp., Sarcocystis spp., and Schistosoma spp. The parasitic diseases which are classified as Th1 -mediated diseases of the invention include both extracellular parasites and obligate intracellular parasites which have at least one stage, and preferably more, of their life cycle that is extracellular. When the parasite is an extracellular parasite having at least one intracellular stage, the invention is useful for treating the parasite while it is in its extracellular stage, and, thus, when it is desirable to produce a Th2 environment.

[0134] In other aspects the method for inducing a Th2 immune response in a subject is useful for generating a Th2 environment. A "Th2 environment" as used herein is a local area of a subject that is characterized by the presence at least one type of Th2-cytokine or a Th2-antibody. Thus the generation of a Th2 environment is characterized by the induction of at least one type of Th2-cytokine or Th2-antibody. In some situations when it is desirable to generate a Th2 environment, the subject has a Th1 mediated disease but in other situations the subject may not have a Th1 mediated disease.

[0135] For example, ocular lesions are extremely common following HSV-1 reactivation and are associated with the infiltration of CD4+ and CD8+ T cells, macrophages, neutrophils and the production of Th 1 cytokines (Rouse, 1996). Thus, a treatment, according to the invention, is the topical administration of Th2 immunostimulatory nucleic acids capable of inducing Th2 cytokines. In a murine model of HSV infection, local treatment with or pre-exposure to Th2 cytokines (IL-10, IL-4, or TGF-.beta.) but not Th1 cytokines (IL-2 or IFN-.gamma.), reduced the severity of ocular lesions associated with HSV (Daheshia et al., 1997, 1998, Chun et al., 1998). Interestingly, intranasal delivery of TGF-.beta. has also been shown to modulate the severity of ocular lesions caused by HSV infection (Kuklin et al., 1998).

[0136] The Th2 immunostimulatory nucleic acids may also be administered topically for the treatment of certain skin conditions. For example, the predominant mechanisms inducing skin lesions in psoriatic patients are thought to be interactions between infiltrating T cells and keratinocytes via the secretion of

the Th1 cytokines IL-2 and IFN-.gamma. the keratinocyte growth factor transforming growth factor alpha (TGF-.alpha.) and the cytokines IL-6 and IL-8. Several anti-psoriatic agents have been identified which act by selective stimulation of Th2 responses (De Jong et al., 1996, Ockenfels et al., 1998). Likewise, since it can selectively stimulate Th2 responses, Th2 immunostimulatory nucleic acids may also be a possible local treatment for Th1 mediated skin disorders.

[0137] The Th2 immunostimulatory nucleic acids may also be administered in conjunction with therapeutic agents, such as adjuvants. Therapeutic agents include but are not limited to systemic and mucosal adjuvants, Th1 or Th2 cytokines, anti-viral agents, anti-bacterial agents, anti-parasitic agents, anti-fungal, and drugs for treating Th1 mediated disorders. Therapeutic agents may be administered directly to the body or may be expressed from an expression system such as a plasmid vector or viral vector.

[0138] Immune responses can be induced and mediated with the co-administration of cytokines with the Th2 immunostimulatory nucleic acids. The term "cytokine" is used as a generic name for a diverse group of soluble proteins and peptides which act as humoral regulators at nano- to picomolar concentrations and which, either under normal or pathological conditions, modulate the functional activities of individual cells and tissues. These proteins also mediate interactions between cells directly and regulate processes taking place in the extracellular environment. Examples of cytokines include, but are not limited to IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-15, granulocyte-macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), interferon-.gamma. (.gamma.-IFN), tumor necrosis factor (TNF), TGF-.beta., FLT-3 ligand, and CD40 ligand.

[0139] A systemic adjuvant is an adjuvant that can be delivered parenterally. Systemic adjuvants include adjuvants that creates a depot effect, adjuvants that stimulate the immune system and adjuvants that do both. An adjuvant that creates a depot effect as used herein is an adjuvant that causes the antigen to be slowly released in the body, thus prolonging the exposure of immune cells to the antigen. This class of adjuvants includes but is not limited to alum (e.g., aluminum hydroxide, aluminum phosphate); or emulsion-based formulations including mineral oil, non-mineral oil, water-in-oil or oil-in-water-in oil emulsion, oil-in-water emulsions such as Seppic ISA series of Montanide adjuvants (e.g., Montanide ISA 720, AirLiquide, Paris, France); MF-59 (a squalene-in-water emulsion stabilized with Span 85 and Tween 80; Chiron Corporation, Emeryville, Calif.; and PROVAX (an oil-in-water emulsion containing a stabilizing detergent and a micelle-forming agent; IDEC, Pharmaceuticals Corporation, San Diego, Calif.).

[0140] Other adjuvants stimulate the immune system, for instance, cause an immune cell to produce and secrete cytokines or IgG. This class of adjuvants includes but is not limited to CpG nucleic acids, saponins purified from the bark of the Q. saponaria tree, such as QS21 (a glycolipid that elutes in the 21.sup.st peak with HPLC fractionation; Aquila Biopharmaceuticals, Inc., Worcester, Me.); poly[di (carboxylatophen- oxy)phosphazene (PCPP polymer; Virus Research Institute, USA); derivatives of lipopolysaccharides such as monophosphoryl lipid A (MPL; Ribi ImmunoChem Research, Inc., Hamilton, Mont.), muramyl dipeptide (MDP; Ribi) andthreonyl-muramyl dipeptide (t-MDP; Ribi); OM-174 (a glucosamine disaccharide related to lipid A; OM Pharma SA, Meyrin, Switzerland); and Leishmania elongation factor (a purified Leishmania protein; Corixa Corporation, Seattle, Wash.).

[0141] Other systemic adjuvants are adjuvants that create a depot effect and stimulate the immune system. These compounds are those compounds which have both of the above-identified functions of systemic adjuvants. This class of adjuvants includes but is not limited to ISCOMs (Immunostimulating complexes which contain mixed saponins, lipids and form virus-sized particles with pores that can hold antigen; CSL, Melbourne, Australia); SB-AS2 (SmithKline Beecham adjuvant system #2 which is an oil-in-water emulsion containing MPL and QS21: SmithKline Beecham Biologicals [SBB], Rixensart,

Belgium); SB-AS4 (SmithKline Beecham adjuvant system #4 which contains alum and MPL; SBB, Belgium); non-ionic block copolymers that form micelles such as CRL 1005 (these contain a linear chain of hydrophobic polyoxpropylene flanked by chains of polyoxyethylene; Vaxcel, Inc., Norcross, Ga.); and Syntex Adjuvant Formulation (SAF, an oil-in-water emulsion containing Tween 80 and a nonionic block copolymer; Syntex Chemicals, Inc., Boulder, Colo.).

[0142] The mucosal adjuvants useful according to the invention are adjuvants that are capable of inducing a mucosal immune response in a subject when administered to a mucosal surface in conjunction with an antigen. Mucosal adjuvants include but are not limited to CpG nucleic acids (e.g. PCT published patent application WO 99/61056), Bacterial toxins: e.g., Cholera toxin (CT), CT derivatives including but not limited to CT B subunit (CTB) (Wu et al., 1998, Tochikubo et al., 1998); CTD53 (Val to Asp) (Fontana et al., 1995); CTK97 (Val to Lys) (Fontana et al., 1995); CTK104 (Tyr to Lys) (Fontana et al., 1995); CTD53/K63 (Val to Asp, Ser to Lys) (Fontana et al., 1995); CTH54 (Arg to His) (Fontana et al., 1995); CTN107 (His to Asn) (Fontana et al., 1995); CTE114 (Ser to Glu) (Fontana et al., 1995); CTE112K (Glu to Lys) (Yamamoto et al., 1997a); CTS61F (Ser to Phe) (Yamamoto et al., 1997a, 1997b); CTS106 (Pro to Lys) (Douce et al., 1997, Fontana et al., 1995); and CTK63 (Ser to Lys) (Douce et al., 1997, Fontana et al., 1995), Zonula occludens toxin, zot, Escherichia coli heat-labile enterotoxin, Labile Toxin (LT), LT derivatives including but not limited to LT B subunit (LTB) (Verweij et al., 1998); LT7K (Arg to Lys) (Komase et al., 1998, Douce et al., 1995); LT61F (Ser to Phe) (Komase et al., 1998); LT112K (Glu to Lys) (Komase et al., 1998); LT118E (Gly to Glu) (Komase et al., 1998); LT146E (Arg to Glu) (Komase et al., 1998); LT192G (Arg to Gly) (Komase et al., 1998); LTK63 (Ser to Lys) (Marchetti et al., 1998, Douce et al., 1997, 1998, Di Tommaso et al., 1996); and LTR72 (Ala to Arg) (Giuliani et al., 1998), Pertussis toxin, PT. (Lycke et al., 1992, Spangler BD, 1992, Freytag and Clemments, 1999, Roberts et al., 1995, Wilson et al., 1995) including PT-9K/129G (Roberts et al., 1995, Cropley et al., 1995); Toxin derivatives (see below) (Holmgren et al., 1993, Verweij et al., 1998, Rappuoli et al., 1995, Freytag and Clements, 1999); Lipid A derivatives (e.g., monophosphoryl lipid A, MPL) (Sasaki et al., 1998, Vancott et al., 1998; Muramyl Dipeptide (MDP) derivatives (Fukushima et al., 1996, Ogawa et al., 1989, Michalek et al., 1983, Morisaki et al., 1983); Bacterial outer membrane proteins (e.g., outer surface protein A (OspA) lipoprotein of Borrelia burgdorferi, outer membrane protine of Neisseria meningitidis)(Marinaro et al., 1999, Van de Verg et al., 1996); Oil-in-water emulsions (e.g., MF59) (Barchfield et al., 1999, Verschoor et al., 1999, O'Hagan, 1998); Aluminum salts (Isaka et al., 1998, 1999); and Saponins (e.g., QS21) Aquila Biopharmaceuticals, Inc., Worster, Me.) (Sasaki et al., 1998, MacNeal et al., 1998), ISCOMs, MF-59 (a squalene-in-water emulsion stabilized with Span 85 and Tween 80; Chiron Corporation, Emeryville, Calif.); the Seppic ISA series of Montanide adjuvants (e.g., Montanide ISA 720; AirLiquide, Paris, France); PROVAX (an oil-in-water emulsion containing a stabilizing detergent and a micell-forming agent; IDEC Pharmaceuticals Corporation, San Diego, Calif.); Syntext Adjuvant Formulation (SAF; Syntex Chemicals, Inc., Boulder, Colo.); poly[di(carboxylatophenoxy)phosphazene (PCPP polymer; Virus Research Institute, USA) and Leishmania elongation factor (Corixa Corporation, Seattle, Wash.).

[0143] Th2 adjuvants include most of the adjuvants listed above, except for CpG nucleic acids. Th1 adjuvants include CpG nucleic acids and MF59, SAF, MPL, and Q521 which under some circumstances, known in the art, induce Th1 -responses.

[0144] Drugs useful for treating Th1 mediated disorders include but are not limited to anti-psoriasis creams, eye or nose drops (e.g., containing cytokines) for herpetic stromal keratitis, Sulfasalazine (i.e., for treating Crohn's disease), glucocorticoids (i.e., Crohn's disease), propylthiouracil (i.e., Grave's disease), methimazole (i.e., Grave's disease), .sup.131I (i.e., Grave's disease), and/or surgery (i.e., Grave's disease), insulin (i.e., IDDM), IFN-.beta.1a (i.e., MS), IFN-.beta.1b (i.e., MS), copolymer 1 (i.e., MS), glucocorticoids (i.e., MS), ACTH (i.e., MS), AVONEX (i.e., MS), glucocorticoids (i.e., pemphigus vulgaris), azathioprine (i.e., pemphigus vulgaris), cyclophosphamide (i.e., pemphigus

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vulgaris), glucocorticoids (i.e., psoriasis), UV-B (i.e., psoriasis), PUVA (i.e., psoriasis), methotrexate (i.e., psoriasis), calcipitriol (i.e., psoriasis), glucocorticoids (i.e., Sjoogren's syndrome), cyclophosphamide (i.e., Sjogren's syndrome), glucocorticoids (i.e., solid organ allograft rejection), OKT3 (i.e., solid organ allograft rejection), FK-506 (i.e., solid organ allograft rejection), cyclosporin A (i.e., solid organ allograft rejection), azathioprine (i.e., solid organ allograft rejection), mycophenolate mofetil (i.e., solid organ allograft rejection), and the following antipsoriatics: Acitretin; Anthralin; Azaribine; Calcipotriene; Cycloheximide; Enazadrem Phosphate; Etretinate; Liarozole Fumarate; Lonapalene; and Tepoxalin.

[0145] Antibacterial agents include but are not limited to Acedapsone; Acetosulfone Sodium; Alamecin; Alexidine; Amdinocillin; Amdinocillin Pivoxil; Amicycline; Amifloxacin; Amifloxacin Mesylate; Amikacin; Amikacin Sulfate; Aminosalicylic acid; Aminosalicylate sodium; Amoxicillin; Amphomycin; Ampicillin; Ampicillin Sodium; Apalcillin Sodium; Apramycin; Aspartocin; Astromicin Sulfate; Avilamycin; Avoparcin; Azithromycin; Azlocillin; Azlocillin Sodium; Bacampicillin Hydrochloride; Bacitracin; Bacitracin Methylene Disalicylate; Bacitracin Zinc; Bambermycins; Benzoylpas Calcium; Berythromycin; Betamicin Sulfate; Biapenem; Biniramycin; Biphenamine Hydrochloride; Bispyrithione Magsulfex; Butikacin; Butirosin Sulfate; Capreomycin Sulfate; Carbadox; Carbenicillin Disodium; Carbenicillin Indanyl Sodium; Carbenicillin Phenyl Sodium; Carbenicillin Potassium; Carumonam Sodium; Cefaclor; Cefadroxil; Cefamandole; Cefamandole Nafate; Cefamandole Sodium; Cefaparole; Cefatrizine; Cefazaflur Sodium; Cefazolin; Cefazolin Sodium; Cefbuperazone; Cefdinir; Cefepime; Cefepime Hydrochloride; Cefetecol; Cefixime; Cefmnenoxime Hydrochloride; Cefmetazole; Cefmetazole Sodium; Cefonicid Monosodium; Cefonicid Sodium; Cefoperazone Sodium; Ceforanide; Cefotaxime Sodium; Cefotetan; Cefotetan Disodium; Cefotiam Hydrochloride; Cefoxitin; Cefoxitin Sodium; Cefpimizole; Cefpimizole Sodium; Cefpiramide; Cefpiramide Sodium; Cefpirome Sulfate; Cefpodoxime Proxetil; Cefprozil; Cefroxadine; Cefsulodin Sodium; Ceftazidime; Ceftibuten; Ceftizoxime Sodium; Ceftriaxone Sodium; Cefuroxime; Cefuroxime Axetil; Cefuroxime Pivoxetil; Cefuroxime Sodium; Cephacetrile Sodium; Cephalexin; Cephalexin Hydrochloride; Cephaloglycin; Cephaloridine; Cephalothin Sodium; Cephapirin Sodium; Cephradine; Cetocycline Hydrochloride; Cetophenicol; Chloramphenicol; Chloramphenicol Palmitate; Chloramphenicol Pantothenate Complex; Chloramphenicol Sodium Succinate; Chlorhexidine Phosphanilate; Chloroxylenol; Chlortetracycline Bisulfate; Chlortetracycline Hydrochloride; Cinoxacin; Ciprofloxacin; Ciprofloxacin Hydrochloride; Cirolemycin; Clarithromycin; Clinafloxacin Hydrochloride; Clindamycin; Clindamycin Hydrochloride; Clindamycin Palmitate Hydrochloride; Clindamycin Phosphate; Clofazimine; Cloxacillin Benzathine; Cloxacillin Sodium; Cloxyquin; Colistimethate Sodium; Colistin Sulfate; Coumermycin; Coumermycin Sodium; Cyclacillin; Cycloserine; Dalfopristin; Dapsone; Daptomycin; Demeclocycline; Demeclocycline Hydrochloride; Demecycline; Denofungin; Diaveridine; Dicloxacillin; Dicloxacillin Sodium; Dihydrostreptomycin Sulfate; Dipyrithione; Dirithromycin; Doxycycline; Doxycycline Calcium; Doxycycline Fosfatex; Doxycycline Hyclate; Droxacin Sodium; Enoxacin; Epicillin; Epitetracycline Hydrochloride; Erythromycin; Erythromycin Acistrate; Erythromycin Estolate; Erythromycin Ethylsuccinate; Erythromycin Gluceptate; Erythromycin Lactobionate; Erythromycin Propionate; Erythromycin Stearate; Ethambutol Hydrochloride; Ethionamide; Fleroxacin; Floxacillin; Fludalanine; Flumequine; Fosfomycin; Fosfomycin Tromethamine; Fumoxicillin; Furazolium Chloride; Furazolium Tartrate; Fusidate Sodium; Fusidic Acid; Gentamicin Sulfate; Gloximonam; Gramicidin; Haloprogin; Hetacillin; Hetacillin Potassium; Hexedine; Ibafloxacin; Imipenem; Isoconazole; Isepamicin; Isoniazid; Josamycin; Kanamycin Sulfate; Kitasamycin; Levofuraltadone; Levopropylcillin Potassium; Lexithromycin; Lincomycin; Lincomycin Hydrochloride; Lomefloxacin; Lomefloxacin Hydrochloride; Lomefloxacin Mesylate; Loracarbef; Mafenide; Meclocycline; Meclocycline Sulfosalicylate; Megalomicin Potassium Phosphate; Mequidox; Meropenem; Methacycline; Methacycline Hydrochloride; Methenamine; Methenamine Hippurate; Methenamine Mandelate; Methicillin Sodium; Metioprim; Metronidazole Hydrochloride; Metronidazole Phosphate; Mezlocillin; Mezlocillin Sodium; Minocycline; Minocycline

Hydrochloride; Mirincamycin Hydrochloride; Monensin; Monensin Sodium; Nafcillin Sodium; Nalidixate Sodium; Nalidixic Acid; Natamycin; Nebramycin; Neomycin Palmitate; Neomycin Sulfate; Neomycin Undecylenate; Netilmicin Sulfate; Neutramycin; Nifuradene; Nifuraldezone; Nifuratel; Nifuratrone; Nifurdazil; Nifurimide; Nifurpirinol; Nifurquinazol; Nifurthiazole; Nitrocycline; Nitrofurantoin; Nitromide; Norfloxacin; Novobiocin Sodium; Ofloxacin; Ormetoprim; Oxacillin Sodium; Oximonam; Oximonam Sodium; Oxolinic Acid; Oxytetracycline; Oxytetracycline Calcium; Oxytetracycline Hydrochloride; Paldimycin; Parachlorophenol; Paulomycin; Pefloxacin; Pefloxacin Mesylate; Penamecillin; Penicillin G Benzathine; Penicillin G Potassium; Penicillin G Procaine; Penicillin G Sodium; Penicillin V; Penicillin V Benzathine; Penicillin V Hydrabamine; Penicillin V Potassium; Pentizidone Sodium; Phenyl Aminosalicylate; Piperacillin Sodium; Pirbenicillin Sodium; Piridicillin Sodium; Pirlimycin Hydrochloride; Pivampicillin Hydrochloride; Pivampicillin Pamoate; Pivampicillin Probenate; Polymyxin B Sulfate; Porfiromycin; Propikacin; Pyrazinamide; Pyrithione Zinc; Quindecamine Acetate; Quinupristin; Racephenicol; Ramoplanin; Ranimycin; Relomycin; Repromicin; Rifabutin; Rifametane; Rifamexil; Rifamide; Rifampin; Rifapentine; Rifaximin; Rolitetracycline; Rolitetracycline Nitrate; Rosaramicin; Rosaramicin Butyrate; Rosaramicin Propionate; Rosaramicin Sodium Phosphate; Rosaramicin Stearate; Rosoxacin; Roxarsone; Roxithromycin; Sancycline; Sanfetrinem Sodium; Sarmoxicillin; Sarpicillin; Scopafingin; Sisomicin; Sisomicin Sulfate; Sparfloxacin; Spectinomycin Hydrochloride; Spiramycin; Stallimycin Hydrochloride; Steffimycin; Streptomycin Sulfate; Streptonicozid; Sulfabenz; Sulfabenzamide; Sulfacetamide; Sulfacetamide Sodium; Sulfacytine; Sulfadiazine; Sulfadiazine Sodium; Sulfadoxine; Sulfalene; Sulfamerazine; Sulfameter; Sulfamethazine; Sulfamethizole; Sulfamethoxazole; Sulfamonomethoxine; Sulfamoxole; Sulfanilate Zinc; Sulfanitran; Sulfasalazine; Sulfasomizole; Sulfathiazole; Sulfazamet; Sulfisoxazole; Sulfisoxazole Acetyl; Sulfisoxazole Diolamine; Sulfomyxin; Sulopenem; Sultamicillin; Suncillin Sodium; Talampicillin Hydrochloride; Teicoplanin; Temafloxacin Hydrochloride; Temocillin; Tetracycline; Tetracycline Hydrochloride; Tetracycline Phosphate Complex; Tetroxoprim; Thiamphenicol; Thiphencillin Potassium; Ticarcillin Cresyl Sodium; Ticarcillin Disodium; Ticarcillin Monosodium; Ticlatone; Tiodonium Chloride; Tobramycin; Tobramycin Sulfate; Tosufloxacin; Trimethoprim; Trimethoprim Sulfate; Trisulfapyrimidines; Troleandomycin; Trospectomycin Sulfate; Tyrothricin; Vancomycin; Vancomycin Hydrochloride; Virginiamycin; Zorbamycin.

[0146] Anti-fungal agents include but are not limited to Acrisorcin; Ambruticin; Amphotericin B; Azaconazole; Azaserine; Basifungin; Bifonazole; Biphenamine Hydrochloride; Bispyrithione Magsulfex; Butoconazole Nitrate; Calcium Undecylenate; Candicidin; Carbol-Fuchsin; Chlordantoin; Ciclopirox; Ciclopirox Olamine; Cilofungin; Cisconazole; Clotrimazole; Cuprimyxin; Denofungin; Dipyrithione; Doconazole; Econazole; Econazole Nitrate; Enilconazole; Ethonam Nitrate; Fenticonazole Nitrate; Filipin; Fluconazole; Flucytosine; Fungimycin; Griseofulvin; Hamycin; Isoconazole; Itraconazole; Kalafungin; Ketoconazole; Lomofingin; Lydimycin; Mepartricin; Miconazole; Miconazole Nitrate; Monensin; Monensin Sodium; Naftifine Hydrochloride; Neomycin Undecylenate; Nifuratel; Nifurmerone; Nitralamine Hydrochloride; Nystatin; Octanoic Acid; Orconazole Nitrate; Oxiconazole Nitrate; Oxifungin Hydrochloride; Parconazole Hydrochloride; Partricin; Potassium Iodide; Proclonol; Pyrithione Zinc; Pyrrolnitrin; Rutamycin; Sanguinarium Chloride; Saperconazole; Scopafungin; Selenium Sulfide; Sinefungin; Sulconazole Nitrate; Terbinafine; Terconazole; Thiram; Ticlatone; Tioconazole; Tolciclate; Tolindate; Tolnaftate; Triacetin; Triafuigin; Undecylenic Acid; Viridoflilvin; Zinc Undecylenate; and Zinoconazole Hydrochloride.

[0147] Anti-parasitic agents include but are not limited to Acedapsone; Amodiaquine Hydrochloride; Amquinate; Arteflene; Chloroquine; Chloroquine Hydrochloride; Chloroquine Phosphate; Cycloguanil Pamoate; Enpiroline Phosphate; Halofantrine Hydrochloride; Hydroxychloroquine Sulfate; Mefloquine Hydrochloride; Menoctone; Mirincamycin Hydrochloride; Primaquine Phosphate; Pyrimethamine; Quinine Sulfate; and Tebuquine.

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[0148] Anti-viral agents include but are not limited to Acemannan; Acyclovir; Acyclovir Sodium; Adefovir; Alovudine; Alvircept Sudotox; Amantadine Hydrochloride; Aranotin; Arildone; Atevirdine Mesylate; Avridine; Cidofovir; Cipamfylline; Cytarabine Hydrochloride; Delavirdine Mesylate; Desciclovir; Didanosine; Disoxaril; Edoxudine; Enviradene; Enviroxime; Famciclovir; Famotine Hydrochloride; Fiacitabine; Fialuridine; Fosarilate; Foscamet Sodium; Fosfonet Sodium; Ganciclovir; Ganciclovir Sodium; Idoxuridine; Kethoxal; Lamivudine; Lobucavir; Memotine Hydrochloride; Methisazone; Nevirapine; Penciclovir; Pirodavir; Ribavirin; Rimantadine Hydrochloride; Saquinavir Mesylate; Somantadine Hydrochloride; Sorivudine; Statolon; Stavudine; Tilorone Hydrochloride; Trifluridine; Valacyclovir Hydrochloride; Vidarabine; Vidarabine Phosphate; Vidarabine Sodium Phosphate; Viroxime; Zalcitabine; Zidovudine; Zinviroxime.

[0149] When the Th2 immunostimulatory nucleic acid is administered in conjunction with antigens and/or therapeutics, the Th2 immunostimulatory nucleic acid can be administered before, after, and/or simultaneously with the antigens and/or therapeutics. For instance, the combination of Th2 immunostimulatory nucleic acid and/or therapeutic may be administered with a priming dose of antigen. Either or both of the Th2 immunostimulatory nucleic acid and/or therapeutic may then be administered with the boost dose. Alternatively, the combination of Th2 immunostimulatory nucleic acid and/or therapeutic may be administered with a boost dose of antigen. Either or both of the of Th2 immunostimulatory nucleic acid and/or therapeutic may then be administered with the prime dose. A "prime dose" is the first dose of antigen administered to the subject. In the case of a subject that has an infection the prime dose may be the initial exposure of the subject to the infectious microbe and thus the combination of Th2 immunostimulatory nucleic acid and/or therapeutic is administered to the subject with the boost dose. A "boost dose" is a second or third, etc, dose of antigen administered to a subject that has already been exposed to the antigen. In some cases the prime dose administered with the combination of Th2 immunostimulatory nucleic acid and/or therapeutic is so effective that a boost dose is not required to protect a subject at risk of infection from being infected. In cases where the combination of Th2 immunostimulatory nucleic acid and/or therapeutic is given without antigen with repeated administrations, the Th2 immunostimulatory nucleic acid and/or therapeutic may be given alone for one or more of the administrations.

[0150] Th2 immunostimulatory nucleic acids also increase antibody dependent cellular cytotoxicity (ADCC). ADCC can be performed using a Th2 immunostimulatory nucleic acid in combination with an antibody specific for a cellular target, such as a cancer cell. When the Th2 immunostimulatory nucleic acid is administered to a subject in conjunction with the antibody the subjects immune system is induced to kill the tumor cell. The antibodies useful in the ADCC procedure include antibodies which interact with a cell in the body. Many such antibodies specific for cellular targets have been described in the art and many are commercially available. These antibodies include but are not limited to those presented in the Table below.

2 Antibody-Based Immune Therapy Product Development (by companies) Clinical. Trial Antibody Phase {tc \.vertline.3 "Cl. Classification Indication Drug Name/Antibody Company(ies) Trial Phase"} 1 non-Hodgkin's Rituxan .TM. (rituximab, IDEC/Genentech, Inc./Hoffinann- Mkt 12/97 (received lymphoma Mabthera) (IDEC-C2B8, La Roche (first monoclonal mkt approval in EU chimeric murine/human anti- antibody licensed for the June 98, CS) CD2O MAb) treatment of cancer in the U.S.) 1 Adjuvant therapy Panorex .RTM. (17-1A) (murine Centocor/Glaxo/Ajinomoto III, expect results mid for colorectal monoclonal antibody) 1998, est. NDA 2001, (Dukes-C) on mkt in Germany 1994 1 Pancreatic, lung, Panorex .RTM. (17-1A) (chimeric Centocor/Ajinomoto III in U.S. and Europe breast, ovary murine monoclonal antibody) non-small cell 3622W94 MAb that binds to Glaxo Wellcome plc II (NCI Phase tin lung, prostate EGP4O (17-lA) combo with IL-2 and (adjuvant) pancarcinoma antigen on GM-CSF) adenocarcinomas 2 Breast/ovarian Herceptin, anti-Her2 bMAb Genentech/Hoffmann-La Roche FDA-approval recommended 2 Renal cell C225 (chimeric monoclonal ImClone Systems II/III

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(12/1997) antibody to epidermal growth factor receptor (EGFr)) 2 Breast C225 (chimeric anti-EGFr ImClone Systems Ib/IIa (3/1996) monoclonal antibody) + taxol 2 prostate C225 (chimeric anti-EGFr ImClone Systems (licensed from Ib/IIa (1/1996) monoclonal antibody) + RPR) doxorubicin 2 prostate C225 (chimeric anti-EGFr ImClone Systems Ib/IIa (1/1996) monoclonal antibody) + adriamycin 3 Small cell lung BEC2 (anti-idiotypic MAb, ImClone Systems III (5/1998) mimics the GD3 epitope) (with BCG?) 3 ? Ovarex (B43.13, anti- Altarex, Canada II/III (1997) idiotypic CA 125, mouse MAb) 3 Melanoma BEC2 (anti-idiotypic MAb, ImClone Systems Ib/IIa mimics the GD3 epitope) 3 Melanoma, small- 4B5 anti-idiotype Ab Novopharm Biotech, Inc. IND filed 9/1997 cell lung 4 Lung, breast, Anti-VEGF, RhuMAb Genentech II prostate, colorectal (inhibits angiogenesis) 5 Breast, ovarian MDX-210 (humanized anti- Medarex/Novartis II (6/1994) HER-2 bispecific antibody) 5 Prostate, non- MDX-210 (humanized anti- Medarex/Novartis II (5/1995) small cell lung, HER-2 bispecific antibody) pancreatic, breast 5 Renal and colon MDX-210 (humanized anti- Medarex/Novartis II HER-2 bispecific antibody) 5 Acute myleoid MDX-22 (humanized Medarex II leukemia bispecific antibody, MAb conjugates) (complement cascade activators) 5 Cancer MDX-210 (humanized anti- Medarex I/II (7/1998) HER-2 bispecific antibody) 5 Lung, colon, MDX-220 (bispecific for Medarex I/II (1998) prostate, ovarian, tumors that express TAG-72) endometrial, pancreatic and gastric 5 Prostate MDX-210 (humanized anti-Medarex/Novartis I/II (8/1996) HER-2 bispecific antibody) 5 EGF receptor MDX-447 (humanized anti-Medarex/Merck KgaA I/II (9/1995) cancers (bead & EGF receptor bispecific neck, prostate, antibody) lung, bladder, cervical, ovarian) 5 Comb. Therapy MDX-210 (humanized anti- Medarex/Novartis I/II (6/1995) with G-CSF for HER-2 bispecific antibody) various cancers, esp. breast 5 Melanoma, MDX-260 bispecific, targets Medarex, Inc. Preclin. glioma, GD-2 neuroblastoma Bone metastases Quadramet (CYT-424) Cytogen Corp. Submitted applic. For radiotherapeutic agent approval in Canada (3/1997), approved for U.S. mkt? non-Hodgkin's IDEC-Y2B8 (murine, anti- IDEC III lymhoma CD20 MAb labeled with Yttrium-90) non-Hodgkin's Oncolym (Lym-1 monoclonal Techniclone International/Alpha II/III (1/1996) lymphoma antibody linked to 131 iodine) Therapeutics Acute myleoid SMART M195 Ab, Protein Design Labs II/III leukemia humanized non-Hodgkin's .sup.131I LYM-1 (Oncolym .TM.) Techniclone II/III lymphoma Corporation/Cambridge Antibody Technology Acute ATRAGEN .RTM. Aronex Pharmaceuticals, Inc. II, to file NDA 1998 promyclocytic leukemia Head & neck, C225 (chimeric anti-EGFr ImClone Systems II/III (1998) non-small cell monoclonal antibody) + lung cancer cisplatin or radiation non-Hodgkin's Bexxar (anti-CD20 Mab Coulter Pharma (Clinical results II/III lymphoma labeled with .sup.131I) have been positive, but the drug has been associated with significant bone marrow toxicity) Kaposi's sarcoma ATRAGEN .RTM. Aronex Pharmaceuticals, Inc. II, completed B cell lymphoma Rituxan .TM. (MAb against IDEC Pharmaceuticals II (clinical trial in CD20) pan-B Ab in combo. Corp./Genentech Germany underway) with chemotherapy Chronic LDP-03, huMAb to the LeukoSite/Hex Oncology II (1998) lymphocytic leukocyte antigen leukemia (CLL) CAMPATH Cancer ior t6 (anti CD6, murine Center of Molecular Immunology IIb MAb) CTCL Acute MDX-11 (complement Medarex II (12/1993) myelogenous activating receptor (CAR) leukemia (AML) monoclonal antibody) Ex vivo bone MDX-11 (complement Medarex II marrow purging in activating receptor (CAR) acute monoclonal antibody) myelogenous leukemia (AML) Ovarian OV103 (Yttrium-90 labelled Cytogen II antibody) Prostate OV103 (Yttrium-90 labelled Cytogen II antibody) non-Hodgkin's ATRAGEN .RTM. Aronex Pharmaceuticals, Inc. II lymphoma Leukemia, Zenapax (SMART Anti-Tac Protein Design Labs II lymphoma (IL-2 receptor) Ab, humanized) Acute SMART M195 Ab, Protein Design Labs II promyclocytic humanized leukemia Melanoma MELIMMUNE-2 (murine IDEC I/II (1993) monoclonal antibody therapeutic vaccine) Melanoma MELIMMUNE-1 (murine IDEC I/II monoclonal antibody therapeutic vaccine) Colorectal and CEACIDE .TM. (1-131) Immunomedics, Inc. I/II other non-Hodgkin's B Pretarget .TM. radioactive NeoRx I(6/1998) cell lymphoma antibodies Cancer NovoMAb-G2 (pancarcinoma Novopharm Biotech, Inc. I in Canada (12/97) specific Ab) Brain TNT (chimeric MAb to Techniclone I (11/97) histone antigens) Corporation/Cambridge Antibody Technology Brain TNT (chimeric MAb to Techniclone I (11/1997) histone antigens) International/Cambridge Antibody Technology Brain, melanomas, Gliomab-H (Monoclonals - Novopharm I (1/1996) neuroblastomas Humanized Abs) Colorectal GNI-250 MAb Genetics

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Institute/AHP I (>1991) Cancer EMD-72000 (chimeric-EUF Merck KgaA I antagonist) non-Hodgkin's B- LymphoCide (humanized Immunomedics I cell lymphoma LL2 antibody) Acute CMA 676 (monoclonal Immunex/AHP I myelogenous antibody conjugate) leukemia Colon, lung, Monopharm-C Novopharm Biotech, Inc. I pancreatic Radioimmuno- egf/r3 (anti EGF-R Center of Molecular Immunology IND filed therapy humanized Ab) Colorectal br c5 (murine MAb Center of Molecular Immunology IND filed colorectal) for radioimmunotherapy Breast cancer BABS (biosynthetic antibody Creative BioMolecules/Chiron Lead/Preclin. binding site) proteins Tumor-associated FLK-2 (monoclonal antibody ImClone Systems/Chugai Lead (1994) angiogenesis to fetal liver kinase-2 (FLK-2)) Small-cell lung Humanized MAb/small-drug ImmunoGen, Inc. Preclin. conjugate Cancer ANA Ab Procyon Biopharma, Inc. Preclin. B-cell lymphoma SMART ID10 Ab Protein Design Labs Preclin. Breast, lung, colon SMART ABL 364 Ab Protein Design Labs/Novartis Preclin. Colorectal ImmuRAIT-CEA Immunomedics, Inc. Pilot

clinicals

[0151] In some embodiments of the invention, the Th2 immunostimulatory nucleic acids are administered to a subject having cancer, or a subject at risk of developing cancer in combination with a therapeutic agent, such as a chemotherapeutic agent. Chemotherapeutic agents include methotrexate, vincristine, adriamycin, cisplatin, non-sugar containing chloroethylnitrosoureas, 5-fluorouracil, mitomycin C, bleomycin, doxorubicin, dacarbazine, taxol, fragyline, Meglamine GLA, valrubicin, carmustaine and poliferposan, MM1270, BAY 12-9566, RAS famesyl transferase inhibitor, famesyl transferase inhibitor, MMP, MTA/LY231514, LY264618/Lometexol, Glamolec, CI-994, TNP-470, Hycamtin/Topotecan, PKC412, Valspodar/PSC833, Novantrone/Mitroxantrone, Metaret/Suramin, Batimastat, E7070, BCH-4556, CS-682, 9-AC, AG3340, AG3433, Incel/VX-710, VX-853, ZDO101, IS1641, ODN 698, TA 2516/Marmistat, BB2516/Marmistat, CDP 845, D2163, PD183805, DX8951f, Lemonal DP 2202, FK 317, Picibanil/OK-432, AD 32/Valrubicin, Metastron/strontium derivative, Temodal/Temozolomide, Evacet/liposomal doxorubicin, Yewtaxan/Placlitaxel, Taxol/Paclitaxel, Xeload/Capecitabine, Furtulon/Doxifluridine, Cyclopax/oral paclitaxel, Oral Taxoid, SPU-077/Cisplatin, HMR 1275/Flavopiridol, CP-358 (774)/EGFR, CP-609 (754)/RAS oncogene inhibitor, BMS-182751/oral platinum, UFT(Tegafur/Uracil), Ergamisol/Levamisole, Eniluracil/776C85/5FU enhancer, Campto/Levamisole, Camptosar/Irinotecan, Tumodex/Ralitrexed, Leustatin/Cladribine, Paxex/Paclitaxel, Doxil/liposomal doxorubicin, Caelyx/liposomal doxorubicin, Fludara/Fludarabine, Pharmarubicin/Epirubicin, DepoCyt, ZD1839, LU 79553/Bis-Naphtalimide, LU 103793/Dolastain, Caetyx/liposomal doxorubicin, Gemzar/Gemcitabine, ZD 0473/Anormed, YM 116, Iodine seeds, CDK4 and CDK2 inhibitors, PARP inhibitors, D4809/Dexifosamide, Ifes/Mesnex/Ifosamide, Vumon/Teniposide, Paraplatin/Carboplatin, Plantinol/cisplatin, Vepeside/Etoposide, ZD 9331, Taxotere/Docetaxel, prodrug of guanine arabinoside, Taxane Analog, nitrosoureas, alkylating agents such as melphelan, cyclophosphamide, Aminoglutethimide, Asparaginase, Busulfan, Carboplatin, Chlorombucil, Cytarabine HCI, Dactinomycin, Daunorubicin HCl, Estramustine phosphate sodium, Etoposide (VP16-213), Floxuridine, Fluorouracil (5-FU), Flutamide, Hydroxyurea (hydroxycarbamide), Ifosfamide, Interferon Alfa-2a, Alfa-2b, Leuprolide acetate (LHRH-releasing factor analogue), Lomustine (CCNU), Mechlorethamine HCl (nitrogen mustard), Mercaptopurine, Mesna, Mitotane (o.p'-DDD), Mitoxantrone HCl, Octreotide, Plicamycin, Procarbazine HCl, Streptozocin, Tamoxifen citrate, Thioguanine, Thiotepa, Vinblastine sulfate, Amsacrine (m-AMSA), Azacitidine, Erthropoietin, Hexamethylmelamine (HMM), Interleukin 2, Mitoguazone (methyl-GAG; methyl glyoxal bis-guanylhydrazone; MGBG), Pentostatin (2'deoxycoformycin), Semustine (methyl-CCNU), Teniposide (VM-26) and Vindesine sulfate.

[0152] Th2 immunostimulatory nucleic acids may also be administered with cancer vaccines selected from the group consisting of EGF, Anti-idiotypic cancer vaccines, Gp75 antigen, GMK melanoma vaccine, MGV ganglioside conjugate vaccine, Her2/neu, Ovarex, M-Vax, O-Vax, L-Vax, STn-KHL

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theratope, BLP25 (MUC-1), liposomal idiotypic vaccine, Melacine, peptide antigen vaccines, toxin/antigen vaccines, MVA-based vaccine, PACIS, BCG vacine, TA-HPV, TA-CIN, DISC-virus and ImmuCyst/TheraCys. Biological response modifiers include interferon, and lymphokines such as IL-2. Hormone replacement therapy includes tamoxifen alone or in combination with progesterone.

[0153] One category of subjects intended for treatment according to the methods of the invention include those that have a cancer or are at risk of developing a cancer selected from the group consisting of basal cell carcinoma, bladder cancer, bone cancer, brain and CNS cancer, breast cancer, cervical cancer, colon and rectum cancer, connective tissue cancer, esophageal cancer, eye cancer, kidney cancer, larynx cancer, liver cancer, lung cancer, Hodgkin's lymphoma, Non-Hodgkin's lymphoma, melanoma, myeloma, leukemia, oral cavity cancer (e.g., lip, tongue, mouth, and pharynx), ovarian cancer, pancreatic cancer, prostate cancer, rhabdomyosarcoma, skin cancer, stomach cancer, testicular cancer, and uterine cancer. In preferred embodiments, the cancer to be treated may be selected from the group consisting of esophageal cancer, eye cancer, larynx cancer, oral cavity cancer (e.g., lip, tongue, mouth, and pharynx), skin cancer, cervical cancer, colon and rectum cancer, eye cancer, melanoma, stomach cancer, and uterine cancer.

[0154] The Th2 immunostimulatory nucleic acids and/or antigens and/or therapeutics may be delivered to the subject using conventional mucosal, local or parenteral routes as long as higher doses are administered when parenteral routes are used. Preferred mucosal routes of administration include but are not limited to oral, intranasal, intratracheal, inhalation, ocular, vaginal, and rectal.

[0155] For oral administration, the compounds (i.e., Th2-immunostimulatory nucleic acid, antigen, other therapeutic agent) can be formulated readily by combining the active compound(s) with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a subject to be treated. Pharmaceutical preparations for oral use can be obtained as solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. Optionally the oral formulations may also be formulated in saline and/or buffers for neutralizing internal acid conditions.

[0156] Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

[0157] Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. Microspheres formulated for oral administration may also be used. Such microspheres have been well defined in the art. All formulations for oral administration should be in dosages suitable for such administration.

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[0158] For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

[0159] For administration by inhalation, the compounds for use according to the present invention may be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g. gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[0160] The compounds may also be formulated in rectal or vaginal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

[0161] In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt. The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

[0162] The compounds may also be administered locally. Compounds are administered locally when they are delivered directly to the site of action. For instance, local administration, includes but is not limited to delivery to the skin to induce antigen-specific immune responses or Th1 mediated skin disorders and direct injection or implantation into the site of a tumor. One preferred form of local administration is direct injection into the site of a tumor for ADCC.

[0163] The compounds of the invention can be administered to the skin, e.g., topically in the form of a skin cream, by injection into the skin, or any other method of administration where access to the skin cells and/or target APCs by the compounds is obtained. In some embodiments, topical administration is preferred, due to the accessibility of the skin and the ease of application. One method for accomplishing topical administration includes transdermal administration, such as iontophoresis. Iontophoretic transmission can be accomplished by using commercially-available patches which deliver a compound continuously through unbroken skin for periods of hours to days to weeks, depending on the particular patch. This method allows for the controlled delivery of the compounds through the skin in relatively high concentrations. One example of an iontophoretic patch is the LECTRO PATCH.TM. sold by General Medical Company of Los Angeles, Calif. The patch provides dosages of different concentrations which can be continuously or periodically administered across the skin using electronic stimulation of reservoirs containing the inhibitors or activators. Transdermal administration also includes needleless delivery methods such as those described in U.S. Pat. No. 5,630,796 and PCT Published Patent application WO99/27961. A needleless syringe is an instrument that delivers a compound transdermally without a conventional needle that pierces the skin. Transdermal delivery also includes intradermal (delivery into the dermis or epidermis), percutaneuos and transmucosal administration. Transmucosal administration is local, for instance, when the compounds are administered by direct injection into the mucosal tissue, i.e., the compounds may be injected into the inside of the cheek. Scarification is scratching of the surface of the skin to break through the epidermal layer before applying the drug.

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[0164] Topical administration also includes epidermal administration which involves the mechanical or chemical irritation of the outermost layer of the epidermis sufficiently to provoke an immune response to the irritant. The irritant attracts APCs to the site of irritation where they can then take up the inhibitor or activator. One example of a mechanical irritant is a tyne-containing device. Such a device contains tynes which irritate the skin and deliver the drug at the same time. For instance, the MONO VACC.RTM. manufactured by Pasteur Merieux of Lyon, France. The device contains a syringe plunger at one end and a tyne disk at the other. The tyne disk supports several narrow diameter tynes which are capable of scratching the outermost layer of epidermal cells. Chemical irritants include, for instance, keratinolytic agents, such as salicylic acid and can be used alone or in conjunction with mechanical irritants.

[0165] The compounds may be in a liquid form. Alternatively, the active compounds may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use or used directly as a powder. A powder as used herein refers to any type of solid dosage form including but not limited to particles, such as crystallized product, lyophilized product, spray coated material etc.

[0166] The compounds, when it is desirable to deliver them parenterally, may be formulated for administration by injection, e.g., by bolus injection or continuous infusion. Injections can be e.g., intravenous, intradermal, subcutaneous, intramuscular, or intraperitoneal. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

[0167] Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

[0168] The Th2 immunostimulatory nucleic acids and/or antigens and/or therapeutics may be administered per se (neat) or in the form of a pharmaceutically acceptable salt. When used in medicine the salts should be pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically acceptable salts thereof. Such salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulphuric, nitric, phosphoric, maleic, acetic, salicylic, p-toluene sulphonic, tartaric, citric, methane sulphonic, formic, malonic, succinic, naphthalene-2-sulphonic, and benzene sulphonic. Also, such salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts of the carboxylic acid group.

[0169] Suitable buffering agents include: acetic acid and a salt (1-2% w/v); citric acid and a salt (1-3% w/v); boric acid and a salt (0.5-2.5% w/v); and phosphoric acid and a salt (0.8-2% w/v). Suitable preservatives include benzalkonium chloride (0.003-0.03% w/v); chlorobutanol (0.3-0.9% w/v); parabens (0.01-0.25% w/v) and thimerosal (0.004-0.02% w/v).

[0170] The pharmaceutical compositions of the invention contain an effective amount of a Th2 immunostimulatory nucleic acid and/or antigen and/or therapeutic optionally included in a pharmaceutically-acceptable carrier. The term "pharmaceutically-acceptable carrier" means one or more

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compatible solid or liquid filler, dilutants or encapsulating substances which are suitable for administration to a human or other vertebrate animal. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions also are capable of being commingled with the compounds of the present invention, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficiency.

[0171] The particular administration routes selected for use in the methods of the invention will depend, of course, upon the particular adjuvants or antigen selected, the particular condition being treated and the dosage required for therapeutic efficacy. The methods of this invention, generally speaking, may be practiced using any mode of administration that is medically acceptable, meaning any mode that produces effective levels of an immune response without causing clinically unacceptable adverse effects. Preferred modes of administration are discussed herein.

[0172] The Th2 immunostimulatory nucleic acid may be directly administered to the subject or may be administered in conjunction with a nucleic acid delivery complex. A "nucleic acid delivery complex" shall mean a nucleic acid molecule associated with (e.g. ionically or covalently bound to; or encapsulated within) a targeting means (e.g. a molecule that results in higher affinity binding to target cell (e.g. dendritic cell surfaces and/or increased cellular uptake by target cells). Examples of nucleic acid delivery complexes include nucleic acids associated with: a sterol (e.g. cholesterol), a lipid (e.g. a cationic lipid, virosome or liposome), or a target cell specific binding agent (e.g. a ligand recognized by target cell specific receptor). Preferred complexes may be sufficiently stable in vivo to prevent significant uncoupling prior to internalization by the target cell. However, the complex can be cleavable under appropriate conditions within the cell so that the nucleic acid is released in a functional form. In some embodiments it is preferred that the nucleic acids that are delivered parenterally are associated with a nucleic acid delivery complex. By targeting the nucleic acids directly to the site of action, lower effective doses of the immunostimulatory nucleic acids can be used. This is especially important for parenteral delivery.

[0173] The compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the compounds into association with a carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing the compounds into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product. Liquid dose units are vials or ampoules. Solid dose units are tablets, capsules and suppositories. For treatment of a patient, depending on activity of the compound, manner of administration, purpose of the immunization (i.e., prophylactic or therapeutic), nature and severity of the disorder, age and body weight of the patient, different doses may be necessary. The administration of a given dose can be carried out both by single administration in the form of an individual dose unit or else several smaller dose units. Multiple administration of doses at specific intervals of weeks or months apart is usual for boosting the antigen-specific responses.

[0174] Other delivery systems can include time-release, delayed release or sustained release delivery systems. Such systems can avoid repeated administrations of the compounds, increasing convenience to the subject and the physician. Many types of release delivery systems are available and known to those of ordinary skill in the art. They include polymer base systems such as poly(lactide-glycolide), copolyoxalates, polycaprolactones, polyesteramides, polyorthoesters, polyhydroxybutyric acid, and polyanhydrides. Microcapsules of the foregoing polymers containing drugs are described in, for example, U.S. Pat. No. 5,075,109. Delivery systems also include non-polymer systems that are: lipids including sterols such as cholesterol, cholesterol esters and fatty acids or neutral fats such as mono-di- and tri-glycerides; hydrogel release systems; sylastic systems; peptide based systems; wax coatings;

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compressed tablets using conventional binders and excipients; partially fused implants; and the like. Specific examples include, but are not limited to: (a) erosional systems in which an agent of the invention is contained in a form within a matrix such as those described in U.S. Pat. Nos. 4,452,775, 4,675,189, and 5,736,152, and (b) diffusional systems in which an active component permeates at a controlled rate from a polymer such as described in U.S. Pat. Nos. 3,854,480, 5,133,974 and 5,407,686. In addition, pump-based hardware delivery systems can be used, some of which are adapted for implantation.

[0175] Other delivery systems useful for administering the Th2 immunostimulatory nucleic acids include, but are not limited to, bioadhesive polymers (Sha et al., 1999), cochleates (Gould-Fogerite et al, 1994, 1996), dendrimers (Kukowska-Latallo et al., 1996, Qin et al, 1998), enteric-coated capsules (Czerkinsky et al., 1987, Levine et al., 1987), emulsomes (Vancott et al., 1998, Lowell et al., 1997), ISCOMs (Mowat et al., 1993, Morein et al., 1999, Hu et al, 1998, Carlsson et al., 1991), liposomes (Childers et al., 1999, Michalek et al, 1989, 1992), microspheres (Gupta et al, 1998, Maloy et al., 1994, Eldridge et al, 1989), nanospheres (Roy et al., 1999), polymer rings (Wyatt et al., 1998), proteosomes (Lowell et al., 1988, 1996) and virosomes (Gluck et al., 1992, Mengiardi et al., 1995, Cryz et al., 1998).

[0176] The term "effective amount" of a Th2 immunostimulatory nucleic acid refers to the amount necessary or sufficient to realize a desired biologic effect. For example, an effective amount of a Th2 immunostimulatory nucleic acid for inducing mucosal immunity is that amount necessary to cause the development of IgA in response to an antigen after exposure to the antigen. The effective amount of a Th2 immunostimulatory nucleic acid for inducing systemic immunity is that amount necessary to cause the development of IgG1 or Th2 cytokines in response to an antigen after exposure to the antigen. Additionally the effective amount of a Th2 immunostimulatory nucleic acid for generating or inducing a Th2 immune response or a Th2 environment is that amount necessary to cause the development of or increase in IgG1 or other Th2 cytokines.

[0177] Combined with the teachings provided herein, by choosing among the various active compounds and weighing factors such as potency, relative bioavailability, patient body weight, severity of adverse side-effects and preferred mode of administration, an effective prophylactic or therapeutic treatment regimen can be planned which does not cause substantial toxicity and yet is entirely effective to treat the particular subject. The effective amount for any particular application can vary depending on such factors as the disease or condition being treated, the particular Th2 immunostimulatory nucleic acid being administered, the antigen, the other therapeutic, the size of the subject, or the severity of the disease or condition. One of ordinary skill in the art can empirically determine the effective amount of a particular Th2 immunostimulatory nucleic acid and/or antigen and/or therapeutic agent without necessitating undue experimentation.

[0178] One important parameter for identifying the effective amount of a Th2 immunostimulatory nucleic acid is the route of delivery. It has been discovered according to the invention that Th2 immunostimulatory nucleic acids administered mucosally or locally are effective in dose ranges which are generally similar to doses of CpG nucleic acids administered through the same routes. Nucleic acids delivered in combination with antigen by parenteral routes generally require higher effective doses to induce antigen specific immune responses. The Th2 immunostimulatory nucleic acids, however, administered parenterally for the purpose of inducing a Th2 immune response or for increasing ADCC or for inducing an antigen specific immune response when the Th2 immunostimulatory nucleic acids are administered in combination with other therapeutic agents or in specialized delivery vehicles are effective in dose ranges which are generally similar to doses of CpG nucleic acids administered through the same routes. In some embodiments higher doses are preferred for parenteral delivery.

[0179] Subject doses of the compounds described herein for mucosal or local delivery typically range

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from about 0.1 .mu.g to 10 mg per administration, which depending on the application could be given daily, weekly, or monthly and any other amount of time therebetween. More typically mucosal or local doses range from about 10 .mu.g to 5 mg per administration, and most typically from about 100 .mu.g to 1 mg, with 2-4 administrations being spaced days or weeks apart. More typically, immune stimulant doses range from 1 .mu.g to 10 mg per administration, and most typically 10 .mu.g to 1 mg, with daily or weekly administrations.

[0180] Subject doses of the compounds described herein for parenteral delivery for the purpose of inducing an antigen-specific immune response, wherein the compounds are delivered with an antigen but not another therapeutic agent can typically be 5 to 10,000 times higher than the effective mucosal dose for vaccine adjuvant or immune stimulant applications, and more typically 10 to 1,000 times higher, and most typically 20 to 100 times higher. In important embodiments, the parenteral dose does not exceed 1 mg/kg per administration. The Th2 immunostimulatory nucleic acids may be administered at even greater doses, for example, at doses approximating 700 mg (i.e., 10 mg/kg) per administration, however, it is recommended that such doses are not administered in a single bolus and are rather administered in a number of administrations or by a number of delivery routes.

[0181] Doses of the compounds described herein for parenteral delivery for the purpose of inducing a Th2 immune response or for increasing ADCC or for inducing an antigen specific immune response when the Th2 immunostimulatory nucleic acids are administered in combination with other therapeutic agents or in specialized delivery vehicles typically range from about 0.1 .mu.g to 10 mg per administration, which depending on the application could be given daily, weekly, or monthly and any other amount of time therebetween. More typically parenteral doses for these purposes range from about 10 .mu.g to 5 mg per administration, and most typically from about 100 .mu.g to 1 mg, with 2-4 administrations being spaced days or weeks apart. In some embodiments, however, parenteral doses for these purposes may be used in a range of 5 to 10,000 times higher than the typical doses described above.

[0182] For any compound described herein the therapeutically effective amount can be initially determined from animal models. A therapeutically effective dose can also be determined from human data for CpG oligonucleotides which have been tested in humans (human clinical trials have been initiated) and for compounds which are known to exhibit similar pharmacological activities, such as other mucosal adjuvants, e.g., LT and other antigens for vaccination purposes, for the mucosal or local administration. Higher doses are required for parenteral administration. The applied dose can be adjusted based on the relative bioavailability and potency of the administered compound. Adjusting the dose to achieve maximal efficacy based on the methods described above and other methods as are well-known in the art is well within the capabilities of the ordinarily skilled artisan.

[0183] In yet another aspect, the invention provides methods for screening nucleic acids for Th2 immunostimulatory activity. Preferably, candidate nucleic acids are tested using the methods described in the Examples. Briefly these methods entail administering to a subject, preferably a murine subject, a nucleic acid optionally with an antigen. Immunoglobulin isotype levels are measured in the subject prior to and following administration of the nucleic acid, as described. In preferred embodiments, the subject does not have above normal levels of Th1 type antibodies or cytokines prior to exposure to the candidate nucleic acid. Nucleic acids that induce the production or increase the level of Th2 type antibodies or cytokines, regardless of their effect on Th1 type antibodies or cytokines level or production can be used as Th2 immunostimulatory nucleic acids. In preferred embodiments, the subject has not been exposed to an infectious agent, especially a bacteria or a virus that carries a Th1 immunostimulatory nucleic acid, and/or does not have an infection by one of these types of microbes.

[0184] The invention will be more fully understood by reference to the following examples. These

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examples, however, are merely intended to illustrate the embodiments of the invention and are not to be construed to limit the scope of the invention. The following examples and the related figures refer to the Th2-immunostimulatory nucleic acid as a non-CpG ODN. For purposes of this patent application the terms "Th2-immunostimulatory nucleic acid" and "non-CpG ODN" are used interchangeably and have the meaning set forth herein for the term "Th2-immunostimulatory nucleic acid."

EXAMPLES

[0185] MATERIALS AND METHODS:

[0186] Immunization of mice: All experiments were carried out using female BALB/c mice aged 6-8 weeks with 5-10 mice per experimental or control group. For all immunizations, mice were lightly anaesthetized with Halothane.RTM. (Halocarbon Laboratories, River Edge, N.J.).

[0187] Antigens: Plasma-derived HBV S protein (HBsAg, ad subtype, Genzyme Diagnostics, San Carlos, Calif.), recombinant HBsAg (ay subtype, Medix Biotech, Foster City, Calif.), formalin-inactivated tetanus toxoid (TT, Pasteur Merieux Connaught, Swiftwater, Pa.), or trivalent influenza virus vaccine (A/Sydney/5/97, A/Beijing/262/95, B/Harbin/7/94, FLUVIRAL.RTM., Biochem Vaccines Inc., Laval, QC, or FLUARIX.RTM., SmithKline Beecham Pharmaceuticals).

[0188] Adjuvants: Non-CpG ODN motifs #1982 (5'-TCCAGGACTTCTCTCAGGTT-3') (SEQ ID NO: 1), #2138 (5'-TCCATGAGCTTCCTGAGCTT-3') (SEQ ID NO: 2), as well as CpG ODN motifs #1826 (TCCATGACGTTCCTGACGTT) (SEQ ID NO: 3) and #2006 (5'-TCGTCGTTTTGTCGTTTTGTCGTT) (SEQ ID NO: 4) were synthesized with nuclease-resistant phosphorothioate backbones by Hybridon (Milford, Mass.). LPS level in ODN was undetectable (<1 ng/mg) by Limulus assay (Whittaker Bioproducts, Walkersville, Md.). Cholera toxin (CT) was obtained from Sigma (St. Louis, Mo.).

[0189] Mucosal immunization of mice: Each animal was immunized with HBsAg (10 or 100 .mu.g), TT (10 or 100 .mu.g), FLUVIRAL.RTM. (50 .mu.l, equivalent to {fraction (1/10)} human dose, contains 1.5 .mu.g A/Sydney/5/97 HA, 1.5 .mu.g A/Beijing/262/95 HA, 1.5 .mu.g B/Harbin/7/94 HA), either alone or in combination with 10, 100 or 500 .mu.g of ODN (CpG or non-CpG) or with 1 or 10 .mu.g CT. Other groups were immunized with a combination vaccine consisting of 10 .mu.g HBsAg, 10 .mu.g TT and 50 .mu.l FLUVIRAL.RTM. with or without the aforementioned adjuvants. For oral immunization, the antigen and adjuvant were made up to a total volume of 50-100 .mu.l with 0.15 M NaCl, and were administered by oral feeding using a 1 c.c. tuberculin syringe (Becton Dickinson, Franklin Lakes, N.J.) attached to a 20-gauge olive tip steel feeding tube (Fine Science Tools Inc., North Vancouver, BC), which was passed through the oral cavity and into the esophagus. For intranasal (IN) immunization, the antigen and adjuvant were made up to a total volume of 5-20 .mu.l with 0.15 M NaCl, which was applied as droplets over both external nares of mice. For intrarectal (IR) immunization, the antigen and adjuvant were made up to a total volume of 20 .mu.l with 0.15 M NaCl and instilled via the anus using a 200 .mu.l pipette tip.

[0190] Intramuscular immunization: Each mouse received a single intramuscular (IM) injection with a 0.3 ml insulin syringe (Becton Dickenson, Franklin Lakes, N.J.) into the left tibialis anterior (TA) muscle of 1 .mu.g HBsAg (ay subtype, Medix Biotech, Foster City, Calif.) or 50 .mu.l FLUARIX.RTM. (equivalent to {fraction (1/10)} human dose, contains 1.5 .mu.g A/Sydney/5/97 HA, 1.5 .mu.g A/Beijing/262/95 HA, 1.5 .mu.g B/Harbin/7/94 HA), without or with 10 or 50 .mu.g adjuvant (non-CpG ODN #1982, CpG ODNs #1826, #2006), made up to a total volume of 60 .mu.l with 0.15 M NaCl.

[0191] Collection of plasma: Plasma was recovered from mice at various times after immunization by

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retro-orbital bleeding and stored at -20.degree. C. until assayed.

[0192] Collection of mucosal samples: Lung washes were carried out on mice 1 wk after third and final immunization. A 0.33 cc Insulin syringe with a 29G1/2 needle attached (Becton Dickenson, Franklin Lakes, N.J.) was used for carrying out lung washes. One ml PBS was drawn into the syringe and a length of polyethylene (PE) tubing that was 1 cm longer than the needle was attached (PE20, ID=0.38 mm, Becton Dickinson). The mouse was killed by anesthetic overdose and the trachea was immediately exposed through an anterior midline incision made using fine-tipped surgical scissors (Fine Science Tools Inc., North Vancouver, BC). A small incision was then made in the trachea and a clamp (Fine Science Tools Inc., North Vancouver, BC) was placed above it. The PE tubing was passed a few mm down the trachea through the incision and a second clamp was placed just below the incision to hold the PE tubing in place in the trachea. The PBS solution was slowly instilled in the lungs then withdrawn three times (80% recovery expected). Recovered samples were centrifuge at 13,000 rpm for 7 min., and the supernatants were collected and stored at -20.degree. C. until assayed by ELISA. Vaginal secretion samples were collected by washing the vaginal cavity three times with 75 .mu.l (225 .mu.l total) of PBS containing 0.1 .mu.g sodium azide (Sigma, St. Louis, Mo.). Saliva was obtained following i.p. injection with 100 .mu.l of 1 mg/ml pilocarpine (Sigma) in PBS to induce saliva flow.

[0193] Evaluation of immune responses

[0194] Systemic humoral response: Antigen-specific antibodies in the mouse plasma were detected and quantified by end-point dilution ELISA assay (in triplicate) for individual animals as described previously (Davis et al., 1998). Briefly, 96-well polystyrene plates (Corning) coated overnight (RT) with HBsAg particles or TT (as used for immunization) (100 .mu.l of 1 or 10 .mu.g/ml for HBsAg and TT respectively, in 0.05 M sodium carbonate-bicarbonate buffer, pH 9.6) were incubated with the plasma for 1 hr at 37.degree. C. Captured antibodies were then detected with horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG, IgG1, IgG2a or IgA (1:4000 in PBS-Tween, 10% FCS: 100 .mu.l/well; Southern Biotechnology Inc., Birmingham, Ala.), followed by addition of o-phenylenediamine dihydrochloride solution (OPD, Sigma), 100 .mu.l/well, for 30 min at RT in the dark. The reaction was stopped by the addition of 4 N H.sub.2SO.sub.4, 50 .mu.l/well. For FLUVIRAL.RTM.- and FLUARIX.RTM.-specific ELISA assays, coating buffer was PBS, and all dilutions subsequent carried in PBS-Tween, 5% FCS.). Each bar represents the group geometric mean (.+-.SEM) of the ELISA end-point dilution titer for the specified antibodies in plasma taken 1-4 weeks after final immunization. Titers were defined as the highest plasma dilution (or saliva, vaginal or lung dilution) resulting in an absorbance value two times that of non-immune plasma (or saliva, vaginal or lung), with a cut-off value of 0.05.

[0195] Mucosal immune responses: This was carried out on recovered saliva or vaginal or lung washes as for plasma (above) except samples were incubated on coated plates for 2 hr at 37.degree. C. and captured antibodies were detected with HRP-conjugated goat anti-mouse IgA (1:1000 in PBS-Tween. 10% PBS: 100 .mu.l/well; Southern Biotechnology Inc). Non-immune saliva, vaginal or lung wash solutions were used to determine negative control values. End-point dilution titers for IgG in plasma and IgA in mucosal samples were defined as the highest sample dilution that resulted in an absorbance value (OD 450) two times greater than that of non-immune, with a cut-off value of 0.05. Antigen-specific Ig titers were shown for individual animals, or in some cases for a group of animals were expressed as geometric mean titers.+-.the standard error of the mean (GMT.+-.SEM) of individual animal values, which were themselves the average of triplicate assays.

[0196] Statistical analysis:

[0197] Data were analyzed using the GraphPAD InStat program (GraphPAD Software, San Diego). The

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statistical significance of the difference between group means was calculated with transformed data (log.sub.10) for ELISA titers by Student's 2-tailed t-test for two groups, or by 1-factor analysis of variance (ANOVA) followed by Tukey's test for three or more groups. Differences were considered to be not significant with $p>0.05$.

[0198] RESULTS

[0199] In FIG. 1 mice were immunized by oral delivery with HBsAg (100 .mu.g) without adjuvant or in combination with CpG ODN (motif #1826, 100 .mu.g), non-CpG ODN (motif #1982, 100 or 500 .mu.g) or Cholera toxin (CT, 10 .mu.g). Each bar represents the group geometric mean (.+-.SEM) of the ELISA end-point dilution titer for HBsAg-specific antibodies (anti-HBs GMT) (Total IgG (FIG. 1a) IgG1 (black bars FIG. 1b) or IgG2a (hatched bars FIG. 1b)) in plasma taken 1 week after final immunization.

[0200] Oral delivery of HBsAg without adjuvant resulted in none or only low anti-HBs IgG titers in the plasma of mice (FIG. 1a). In contrast, much higher levels of anti-HBs IgG antibodies were detected when CpG ODN #1826 (100 .mu.g), CT (10 .mu.g) or non-CpG ODN #1982 (100 or 500 .mu.g) were added ($p<0.05$). Compared to results obtained with CT (10 .mu.g), a classical mucosal adjuvant, HBsAg-specific IgG titers with 100 or 500 .mu.g non-CpG ODN were better (100 .mu.g non-CpG ODN, $p<0.05$) or equally good (500 .mu.g non-CpG ODN, $p>0.05$). Surprisingly, there was no significant difference between results obtained with an equivalent dose (100 .mu.g) of non-CpG and CpG ODN ($p>0.05$). When antibody isotypes were used as an indication of the Th-bias of the responses induced by the different formulations, the addition of non-CpG ODN augmented both IgG1 (Th2-like) and IgG2a (Th1-like) but with a predominance of IgG1 (FIG. 1b), as did CT. In contrast, CpG ODN induced an equally mixed Th1/Th2 response, which is much more Th1-biased than is obtained with HBsAg alone (by other routes, where it is effective on its own).

[0201] Our findings that oral delivery of HBsAg resulted in enhanced IgG levels with both CpG and non-CpG ODN were particularly surprising since we had previously demonstrated, with IM delivery, an enhancement of immune responses with CpG ODN but not non-CpG ODN (FIG. 2) (Davis et al., 1998). In FIG. 2 mice were immunized by intramuscular (IM) injection with 1 .mu.g HBsAg without adjuvant or with 10 .mu.g of CpG ODN (motif #1826) or non-CpG ODN (motif #1982). Each bar represents the group mean (.+-.SEM) of the ELISA end-point dilution titer for HBsAg-specific antibodies (anti-HBs) (total (FIG. 2a) or IgG1 (hatched bars FIG. 2b) or IgG2a (grey bars FIG. 2b)) in plasma taken 4 weeks after immunization.

[0202] When TT was used as antigen for oral delivery, TT-specific total IgG titers in plasma were similarly increased with both CpG ODN and non-CpG ODN, as long as a low enough dose of TT was used. In FIG. 3 mice were immunized by oral delivery on days 0, 7 and 14 with TT (100 .mu.g) without adjuvant or in combination with CpG ODN (motif #1826, 100 .mu.g), non-CpG ODN (motif #1982, 100 or 500 .mu.g) or Cholera toxin (CT, 10 .mu.g). Each bar represents the group geometric mean (.+-.SEM) of the ELISA end-point dilution titer for TT-specific antibodies (anti-TT GMT) (Total IgG (FIG. 3a) IgG1 (black bars FIG. 3b) or IgG2a (hatched bars FIG. 3b)) in plasma taken 1 week after final immunization.

[0203] Thus while an effect for CpG ODN but not non-CpG ODN was seen with a very high 100 .mu.g dose of TT (FIG. 3a), both ODN were effective with a 10 .mu.g dose (see FIGS. 6, 8 and 10). Regardless of TT dose however, antibody isotypes indicated that CpG ODN overcame the strong Th2-bias of the antigen, whereas, responses with both non-CpG ODN or CT remained Th2 (IgG1>>IgG2a) (FIG. 3b).

[0204] FLUVIRAL.RTM. was used as antigen for oral delivery in FIG. 4. In FIG. 4 mice were

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immunized by oral delivery on days 0, 7 and 14 with FLUVIRAL.RTM. (50 .mu.l, {fraction (1/10)} human dose) without adjuvant or in combination with 10 .mu.g of CpG ODN (motif #1826) or non-CpG ODN (motif #2138 or #1982). Each bar represents the group geometric mean (.+-.SEM) of the ELISA end-point dilution titer for FLUVIRAL.RTM.-specific antibodies (anti-FLUVIRAL.RTM. GMT) (Total IgG (FIG. 4a) IgG1 (hatched bars FIG. 4b) or IgG2a (black bars FIG. 4b)) in plasma taken 1 week after final immunization. When FLUVIRAL.RTM. was used as antigen for oral delivery, mean FLUVIRAL.RTM.-specific IgG titers in plasma were augmented similarly (approximately 5-fold) with both non-CpG ODNs (#2138 and #1982) and CpG ODN (#1826) (FIG. 4a). However, whereas the addition of CpG ODN augmented predominantly IgG2a (Th-1 like) antibodies and therefore overcame the strong Th-2 bias of FLUVIRAL.RTM. alone, the non-CpG ODN augmented both IgG1 and IgG2a such that the Th2 bias was retained (FIG. 4b).

[0205] Similar to our findings with HBsAg (FIG. 2), when a similar influenza virus vaccine (FLUARIX.RTM.) was administered IM, no augmentation of Antigen-specific IgG was seen with non-CpG ODN (FIG. 5), indicating that the immunostimulatory properties of non-CpG ODN are associated with mucosal but not parenteral delivery, at least at low concentrations. In FIG. 5 mice were immunized by intramuscular (IM) injection with FLUARIX.RTM. (50 .mu.l, {fraction (1/10)} human dose) without adjuvant or in combination with 50 .mu.g of CpG ODN (motif #2006) or non-CpG ODN (motif#1982). Each bar represents the group mean (.+-.SEM) of the ELISA end-point dilution titer for FLUARIX.RTM.-specific antibodies (anti-FLUARIX.RTM.) in plasma taken 2 weeks after immunization.

[0206] In order to determine whether similar effects would be seen with a multivalent vaccine, mice were immunized orally with a combination of HBsAg/TT/FLUVIRAL.RTM. alone or with CpG (#1826) or non-CpG (#1982) ODN. In FIG. 6 mice were immunized by oral delivery on days 0, 7 and 14 with a combination of HBsAg/TT/FLUVIRAL.RTM. (10 .mu.g, 10 .mu.g, 50 .mu.l respectively) without adjuvant or in combination with 10 .mu.g CpG ODN (motif #1826), or non-CpG ODN (motif #1982). Each symbol represents the ELISA end-point dilution titer for HBsAg-specific (FIG. 6a), TT-specific (FIG. 6b), or FLUVIRAL.RTM.-specific (FIG. 6c) antibodies in plasma of individual mice taken 1 week after final immunization with multiple antigens (HBsAg/TT/FLUVIRAL.RTM., filled circles) or with a single antigen (TT (FIG. 6b) or FLUVIRAL.RTM. (FIG. 6c), filled triangles). Horizontal bars represent the group geometric mean.

[0207] Oral delivery of HBsAg/TT/FLUVIRAL.RTM. without adjuvant resulted in no detectable HBsAg-specific IgG in the plasma of mice and mean TT- and FLUVIRAL.RTM.-specific IgG titers were .about.1000 and 100 respectively (FIG. 6). In contrast, when CpG or non-CpG ODN was added mean TT- and FLUVIRAL.RTM.-specific IgG titers were raised .about.10- to 20-fold and HBsAg-specific IgG was now detected. The combination of different antigens did not result in any competitive inhibition since Antigen-specific titers attained with multiple antigens were as high as those attained with single antigens (FIG. 6b and c, triangle symbols).

[0208] As we had seen with single antigens, the addition of CpG ODN enhanced Th1-like responses (IgG2a>>IgG1), whereas with non-CpG, Th2-like responses were enhanced (IgG1>>IgG2a) (FIG. 7). In FIG. 7 mice were immunized by oral delivery on days 0, 7 and 14 with a combination of HBsAg/TT/FLUVIRAL.RTM. (10 .mu.g, 10 .mu.g, 50 .mu.l respectively) without adjuvant or in combination with 10 .mu.g CpG ODN (motif # 1826), or non-CpG ODN (motif #1982). Each bar represents the group geometric mean of the ELISA end-point dilution titer for FLUVIRAL.RTM.-specific (FIG. 7a) or TT-specific (FIG. 7b) antibodies of IgG1 (grey bars) or IgG2a (black bars) isotypes in plasma taken 1 week after final immunization. Titers were defined as the highest plasma dilution resulting in an absorbance value two times that of non-immune plasma, with a cut-off value of 0.05.

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[0209] In order to determine whether non-CpG ODN would also have stimulatory effects when delivered by different mucosal routes, mice were immunized with TT (10 .mu.g) either alone, or with CpG or non-CpG ODN (100 .mu.g) as adjuvant by intrarectal (IR, FIG. 8a), intranasal (IN, FIG. 8b and FIG. 9) as well as oral routes (FIG. 8c). In addition, control mice were immunized using CT, a conventional mucosal adjuvant (FIG. 8). In FIG. 8 CpG ODN (motif#1826, 100 .mu.g), non-CpG ODN (motif#1982, 100 .mu.g) or Cholera toxin (CT, 10 .mu.g) were used as adjuvant and in FIG. 9 with CpG ODN (motif #1826, 10 or 100 .mu.g) or non-CpG ODN (motif #1982, 100 .mu.g) were used as adjuvant. Each filled circle in FIG. 8 represents the ELISA end-point dilution titer for TT-specific antibodies in plasma of individual mice taken 1 week after final immunization. Grey bars represent the group geometric mean. Each bar in FIG. 9 represents the group geometric mean (.+-.SEM) of the ELISA end-point dilution titer for TT-specific antibodies (anti-TT GMT) of Total IgG (FIG. 9a) or IgG1 (grey bars) or IgG2a (hatched bars) isotypes (FIG. 9b) in plasma taken 1 week after final immunization.

[0210] Non-CpG ODN was found to have a stimulatory effect when delivered by all mucosal routes tested. Delivery of TT by the IR route resulted in {fraction (0/5)}, {fraction (8/10)}, 2/5 and {fraction (5/5)} mice responding (anti-TT IgG in plasma>100) for no adjuvant, CpG ODN, non-CpG ODN and CT respectively; by the IN route resulted in {fraction (0/10)}, {fraction (10/10)}, {fraction (5/5)} and 5/5 mice responding for no adjuvant, CpG ODN, non-CpG ODN and CT respectively; and for oral delivery resulted in {fraction (5/10)}, {fraction (8/9)}, 4/5 and {fraction (5/5)} mice responding for no adjuvant, CpG ODN, non-CpG ODN and CT respectively (FIG. 8). Similar to our findings with oral delivery, when non-CpG ODN were administered by IN delivery an equivalent response was induced to that with CpG ODN or CT ($p<0.05$) (FIG. 8 and FIG. 9a), however, the response with non-CpG ODN was more Th2-like (IgG1>IgG2a) than with CpG ODN (IgG1=IgG2a) (FIG. 9b).

[0211] In FIG. 10 mice were immunized by oral delivery on days 0, 7 and 14 with TT (10 .mu.g) without adjuvant or in combination with CpG ODN (motif #1826, 10 or 100 .mu.g) or non-CpG ODN (motif #1982, 10 or 100 .mu.g). Each bar represents the group geometric mean SEM) of the ELISA end-point dilution titer for TT-specific antibodies (anti-TT GMT) of Total (FIG. 10a) or IgG1 (grey bars) or IgG2a (hatched bars) isotypes (FIG. 10b) in plasma taken 1 week after final immunization. The immunostimulatory effects of non-CpG ODN after oral delivery were observed at both low (10 .quadrature.g) and high (100 .mu.g) doses of non-CpG ODN (FIG. 10a), and, in contrast to CpG DNA, increasing the dose of non-CpG ODN did not alter the IgG2a to IgG1 ratio (FIG. 10b).

[0212] In addition to augmenting systemic immune responses (IgG), non-CpG ODN was also found to augment antigen-specific mucosal immunity (IgA) at a number of mucosal sites. This was found with administration of single antigens, namely HBsAg (FIG. 11), TT (FIG. 12), and FLUVIRAL.RTM. (FIG. 13), or multiple antigens, namely HBsAg/TT/FLUVIRAL.RTM. (FIG. 14). These findings are important since secretory IgA is thought to protect against pathogen entry to the body via a mucosal surface.

[0213] In FIG. 11 mice were immunized by oral delivery on days 0, 7 and 14 with HBsAg (100 .mu.g) without adjuvant or in combination with CpG ODN (motif #1826, 100 or 500 .mu.g), or non-CpG ODN (motif #1982, 100 or 500 .mu.g). Each bar represents the ELISA end-point dilution titer for HBsAg-specific IgA antibodies (anti-HBs IgA) in saliva (FIG. 11a), vaginal washes (FIG. 11b), or lung washes (FIG. 11c) taken 1 week after final immunization and pooled for each group.

[0214] Mice were immunized, in FIG. 12, by oral delivery on days 0, 7 and 14 with TT (100 .mu.g) without adjuvant or in combination with CpG ODN (motif #1826, 100 or 500 .mu.g), non-CpG ODN (motif #1982, 100 or 500 .mu.g) or Cholera toxin (CT, 10 .mu.g). Each bar represents the ELISA end-point dilution titer for TT-specific IgA antibodies (anti-TT IgA) in vaginal washes collected 1 week after final immunization and pooled for each group.

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[0215] In FIG. 13 mice were immunized by oral delivery on days 0, 7 and 14 with FLUVIRAL.RTM. (50 .mu.l, {fraction (1/10)} human dose) without adjuvant or in combination with 10 .mu.g of CpG ODN (motif#1826) or non-CpG ODN (motif #2138). Each filled circle represents the ELISA end-point dilution titer for FLUVIRAL.RTM.-specific IgA antibodies (anti-FLUVIRAL.RTM. IgA) for individual mice in lung washes (FIG. 13a), vaginal washes (FIG. 13b), or saliva (FIG. 13c) taken 1 week after final immunization. Grey and black bars in FIGS. 13b and 13c represent identical treatments given to two separate groups of animals.

[0216] In FIG. 14 mice were immunized by oral delivery on days 0, 7 and 14 with a combination of HBsAg/TT/FLUVIRAL.RTM. (10 .mu.g, 10 .mu.g, 50 .mu.l respectively) without adjuvant or in combination with 10 .mu.g CpG ODN (motif #1826), or non-CpG ODN (motif #1982). Each symbol represents the ELISA end-point dilution titer for HBsAg-specific IgA (FIG. 14b), TT-specific (FIG. 14a), or FLUVIRAL.RTM.-specific (FIG. 14c) antibodies in lung washes of individual mice taken 1 week after final immunization.

[0217] Each of the foregoing patents, patent applications and references that are recited in this application are herein incorporated in their entirety by reference. Having described the presently preferred embodiments, and in accordance with the present invention, it is believed that other modifications, variations and changes will be suggested to those skilled in the art in view of the teachings set forth herein. It is, therefore, to be understood that all such variations, modifications, and changes are believed to fall within the scope of the present invention as defined by the appended claims.

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United States Patent Application *20030092663*
Kind Code **A1**
Raz, Eyal **May 15, 2003**

Immunization-free methods for treating antigen-stimulated inflammation in a mammalian host and shifting the host's antigen immune responsiveness to a Th1 phenotype

Abstract

The invention relates to methods for preventing or reducing antigen-stimulated, granulocytemediated inflammation in tissue of an antigen-sensitized mammal host by delivering an immunostimulatory oligonucleotide to the host. In addition, methods for using the immunostimulatory oligonucleotides to boost a mammal host's immune responsiveness to a sensitizing antigen (without immunization of the host by the antigen) and shifting the host's immune responsiveness to a Th1 phenotype to achieve various therapeutic ends are provided. Kits for practicing the methods of the invention are also provided.

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Serial No.: **229208**
Series Code: **10**
Filed: **August 26, 2002**

U.S. Current Class: **514/44**; 424/178.1; 514/26; 514/8
U.S. Class at Publication: **514/44**; 514/8; 424/178.1; 514/26
Intern'l Class: A61K 048/00

Goverment Interests

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[0001] This invention was made with Government support under Grant No. AI37305, awarded by the National Institutes of Health. The Government may have certain rights in this invention.

Claims

The invention claimed is:

1. A method for preventing or reducing antigen-stimulated, granulocyte-mediated inflammation in a tissue of an antigen-sensitized mammalian host comprising delivering an immunostimulatory oligonucleotide (ISS-ODN) to the host; wherein a reduction in, or the absence of, a Th2 type immune response measured in the host; or a reduction in, or the absence of other clinical signs of inflammation in the host after antigen challenge, indicates that the desired prevention or reduction in granulocyte-mediated inflammation has been achieved.

2. The method according to claim 1 wherein the ISS-ODN includes a hexameric nucleotide sequence consisting of 5'-Purine-Purine-[C]-[G]-Pyri- midine-Pyrimidine-3'.

3. The method according to claim 2 wherein the hexameric nucleotide sequence consists of AACGTT.

4. The method according to claim 2 wherein the hexameric nucleotide sequence is selected from the group of sequences consisting of AGCGTC, GACGTT, GGCGTT, AACGTC, AGCGTC, GACGTC, GGCGTC, AACGCC, AGCGCC, GACGCC, GGCGCC, AGCGCT, GACGCT, GGCGCT, TTCGAA, GGCGTT and AACGCC.

5. The method according to claim 1 wherein the ISS-ODN is conjugated to an immunostimulatory or anti-inflammatory partner selected from the group consisting of non-antigenic polypeptides, antigenic polypeptides, polysaccharides, antibodies, glycoproteins, lipids and steroids.

6. The method according to claim 1 wherein the host is suffering from an inflammatory condition induced by the sensitizing antigen selected from the group of inflammatory conditions consisting of asthma, nasal polyposis, allergic rhinitis, atopic dermatitis, allergic conjunctivitis, eosinophilic fasciitis, ideopathic hypereosinophilic syndrome and cutaneous basophil hypersensitivity.

7. The method according to claim 1 wherein the inflamed tissue is skin or mucosa.

8. The method according to claim 7 wherein the inflamed tissue is respiratory tissue.

9. The method according to claim 8 wherein the host is suffering from asthma.

10. A method for boosting the immune responsiveness of a mammalian host to a sensitizing antigen without immunization of the host by the sensitizing antigen comprising delivering an immunostimulatory oligonucleotide (ISS-ODN) to the host to the host, wherein an increase in the magnitude of the host immune response to the sensitizing antigen indicates that the desired boost to the host immune responsiveness has been achieved.

11. The method according to claim 10 wherein the IS S-ODN includes a hexameric nucleotide sequence consisting of 5'-Purine-Purine-[C]-[G]-Pyri- midine-Pyrimidine-3'.

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12. The method according to claim 11 wherein the hexameric nucleotide sequence consists of AACGTT.

13. The method according to claim 1 wherein the hexameric nucleotide sequence is selected from the group of sequences consisting of AGCGTC, GACGTT, GGCGTT, AACGTC, AGCGTC, GACGTC, GGCGTC, AACGCC, AGCGCC, GACGCC, GGCGCC, AGCGCT, GACGCT, GGCGCT, TTCGAA, GGCGTT and AACGCC.

14. The method according to claim 10 wherein the ISS-ODN is conjugated to an immunostimulatory or anti-inflammatory partner selected from the group consisting of non-antigenic polypeptides, antigenic polypeptides, polysaccharides, antibodies, glycoproteins, lipids and steroids.

15. The method according to claim 10 wherein the host is suffering from an inflammatory condition induced by the sensitizing antigen selected from the group of inflammatory conditions consisting of asthma, nasal polyposis, allergic rhinitis, atopic dermatitis, allergic conjunctivitis, eosinophilic fasciitis, ideopathic hypereosinophilic syndrome and cutaneous basophil hypersensitivity.

16. The method according to claim 10 wherein the inflamed tissue is skin or mucosa.

17. The method according to claim 16 wherein the inflamed tissue is respiratory tissue.

18. The method according to claim 17 wherein the host is suffering from asthma and the host's immune responsiveness to a respiratory allergen is boosted.

19. The method according to claim 10 wherein the antigen is presented by a pathogen and the host's immune responsiveness to an intracellular infection by the pathogen is boosted.

20. The method according to claim 19 wherein the pathogen is a virus.

21. A method for shifting the immune response of a mammal host to a sensitizing antigen toward a Th1 phenotype comprising delivering an immunostimulatory oligonucleotide (ISS-ODN) to the host, wherein detection of a Th1 type immune response by the host indicates that the desired shift to the Th1 phenotype has been achieved.

22. The method according to claim 21 wherein the ISS-ODN includes a hexameric nucleotide sequence consisting of 5'-Purine-Purine-[C]-[G]-Pyri- midine-Pyrimidine-3'.

23. The method according to claim 22 wherein the hexameric nucleotide sequence consists of AACGTT.

24. The method according to claim 22 wherein the hexameric nucleotide sequence is selected from the group of sequences consisting of AACGTT, AGCGTC, GACGTT, GGCGTT, AACGTC, AGCGTC, GACGTC, GGCGTC, AACGCC, AGCGCC, GACGCC, GGCGCC, AGCGCT, GACGCT, GGCGCT, TTCGAA, AACGTT, GGCGTT and AACGCC.

25. The method according to claim 21 wherein the ISS-ODN is conjugated to an immunostimulatory or anti-inflammatory partner selected from the group consisting of non-antigenic polypeptides, antigenic polypeptides, polysaccharides, antibodies, glycoproteins, lipids and steroids.

26. The method according to claim 21 wherein the host is suffering from an inflammatory condition

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induced by the sensitizing antigen selected from the group of inflammatory conditions consisting of asthma, nasal polyposis, allergic rhinitis, atopic dermatitis, allergic conjunctivitis, eosinophilic fasciitis, ideopathic hypereosinophilic syndrome and cutaneous basophil hypersensitivity, and the shift to the Th1 phenotype reduces granulocyte-mediated inflammation in the affected host tissue.

27. The method according to claim 26 wherein the inflamed tissue is skin or mucosa.

28. The method according to claim 27 wherein the inflamed tissue is respiratory tissue.

29. The method according to claim 28 wherein the host is suffering from asthma and the shift to the Th1 phenotype reduces eosinophil infiltration of the host lung tissue.

30. The method according to claim 21 wherein the host is suffering from an intracellular infection by a pathogen and the shift to the Th1 phenotype strengthens the host immune response to the pathogen.

31. The method according to claim 30 wherein the pathogen is a virus.

32. The method according to claim 21 wherein the host is suffering from reduced blood flow to a tissue and the shift to the Th1 phenotype stimulates angiogenesis in the treated tissue.

33. The method according to claim 32 wherein the host is suffering from diabetic retinopathy.

34. The method according to claims 1, 10 or 21 wherein the desired result is measured by determining any of the following values in a sample containing lymphocytes obtained from the ISS-ODN treated host: (1) a reduction in levels of IL-4, IL-5 and/or IL-10 measured before and after antigen challenge or detection of lower levels of IL-4, IL-5 and/or IL-10 in the IS S-ODN treated host as compared to an antigen-challenged control; (2) an increase in levels of IL-12, IL-18 and/or IFN (.alpha., .beta. or .gamma.) before and after antigen challenge or detection of higher levels of IL-12, IL-18 and/or IFN (.alpha., .beta. or .gamma.) in the ISS ODN treated host as compared to an antigen-challenged control; (3) IgG2a antibody production in the ISS-ODN treated host; or (4) a reduction in levels of antigen-specific IgE as measured before and after antigen challenge or detection of lower levels of antigen-specific IgE in the ISS-ODN treated host as compared to an antigen-challenged control.

35. The method according to claims 1 or 10 wherein reduction or suppression of inflammation is measured by assaying inflammatory infiltrate from the host for a reduction in granulocyte counts in inflammatory infiltrate of an affected host tissue as measured in an antigen challenged host before and after ISS-ODN administration or detection of lower levels of granulocyte counts in an ISS-ODN treated host as compared to an antigen-challenged control.

36. A kit for use in-reducing or preventing inflammation in an antigen-sensitized host tissue, as well as in boosting the immune responsiveness of a host to a sensitizing antigen, comprising an immunostimulatory oligonucleotide (ISS-ODN) in a sterile vial, a device for delivering the ISS-ODN directly into a host tissue and at least one assay reagent for use in measuring any of the following values as indicators that the desired reduction or prevention of inflammation or boost in immune responsiveness has been achieved in an ISS-ODN treated host: (1) a reduction in levels of IL-4, IL-5 and/or IL-10 measured before and after antigen challenge; or detection of lower (or absent) levels of IL-4, IL-5 and/or IL-10 in an ISS-ODN treated host as compared to an antigen-primed, or primed and challenged, control; (2) an increase in levels of IL-12, IL-18 and/or IFN (.alpha., .beta. or .gamma.) before and after antigen challenge; or detection of higher levels of IL-12, IL-18 and/or IFN (.alpha., .beta. or .gamma.) in an ISS ODN treated host as compared to an antigen-primed, or primed and challenged, control; (3) IgG2a antibody production in an ISS-ODN treated host; or (4) a reduction in

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levels of antigen-specific IgE as measured before and after antigen challenge; or detection of lower (or absent) levels of antigen-specific IgE in an ISS-ODN treated host as compared to an antigen-primed, or primed and challenged, control.

37. A kit for use in reducing or preventing inflammation in an antigen-sensitized host tissue comprising an immunostimulatory oligonucleotide (IS S-ODN) in a sterile vial, a device for delivering the ISS-ODN directly into a host tissue and at least one assay reagent for use in measuring lymphocyte proliferation, IgG2a antibody levels, serum cytokine levels and/or granuloctye counts in inflammatory infiltrate of an affected host tissue.

Description

FIELD OF THE INVENTION

[0002] The invention relates to methods and oligonucleotide compositions for use in reducing or suppressing granulocyte-mediated inflammation in a host tissue and in modulating the host's immune responsiveness to an antigen.

HISTORY OF THE RELATED ART

[0003] In vertebrates, endothelial cell adhesion by granulocytes (eosinophils, basophils, neutrophils and mast cells) is followed by the release of inflammatory mediators, such as leukotrienes, major basic protein and histamine. In susceptible individuals, the resulting inflammation can damage affected host tissues.

[0004] The most common pathologic inflammatory condition is asthma, which is characterized by marked eosinophil infiltration into respiratory airways, followed by inflammation-induced tissue damage. Other pathologic inflammatory conditions associated with granulocyte infiltration into affected tissues include nasal polyposis, allergic rhinitis, allergic conjunctivitis, atopic dermatitis, eosinophilic fasciitis, idiopathic hypereosinophilic syndrome and cutaneous basophil hypersensitivity, as well as inflammation and fibrosis resulting from increased production of granulocyte-stimulatory cytokines, such as interleukin (IL)-5 and certain interferons (INF).

[0005] Routine treatment of such conditions is typically directed toward inhibiting the activity of inflammatory mediators released after granulocyte adhesion to endothelia (e.g., by delivering a corticoid composition to the affected tissues). Where the identity of an inflammation inducing antigen is known, some immune protection against further antigen challenge can be provided through immunization. However, although effective in stimulating production of neutralizing antibodies, canonical immunization does not effectively stimulate longer term cellular immunity. Moreover, antigen immunization stimulates host production of IL-4 and IL-5. IL-5 encourages granulocyte adhesion to endothelia while IL-4 induces immunoglobulin switching to the IgE isotype at the risk of anaphylaxis.

SUMMARY OF THE INVENTION

[0006] The invention provides means to rapidly suppress antigen-stimulated inflammation in a mammalian host by suppressing granulocyte infiltration into a host tissue. The invention also provides immunization-free means to provide protection to an antigen-sensitized mammalian host against subsequent antigen challenge without risk of anaphylaxis. These aims are achieved by the invention through delivery of an immunostimulatory oligonucleotide (ISS-ODN) to the host without codelivery of

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an immunizing antigen.

[0007] Surprisingly, ISS-ODN have anti-inflammatory properties in addition to their immunostimulatory properties. ISS-ODN are therefore useful in the treatment and prevention of inflammation associated with antigen-stimulated granulocyte infiltration of tissue, such as occurs in the respiratory passages of asthmatics during an asthma attack. Advantageously, delivery of ISS-ODN according to the invention suppresses antigen-stimulated granulocyte infiltration into host tissue even before the ISS-ODN affect the host's immune response to the antigen. Thus, the invention provides an antigen-independent method to reduce antigen-stimulated inflammation by suppressing cellular adhesion, thereby avoiding the release of inflammatory mediators which would be stimulated through granulocyte-binding of endothelial cells.

[0008] An example of a therapeutic application for the invention is in the control of asthma, whereby the ISS-ODN are delivered into pulmonary tissue intranasally or by systemic routes. In asthmatics, eosinophil infiltration of lung tissue occurs mainly during the late phase of an allergic response to a respiratory allergen. Canonical immunotherapy can modulate the host immune response to the allergen and eventually stem the tide of eosinophils into the host airways. However, practice of the invention suppresses eosinophil infiltration of host airways well before the host immune system responds to the respiratory allergen, thereby providing a form of protection against the airway narrowing and respiratory tissue damage which characterize an acute asthma attack.

[0009] In another aspect, the invention provides means to shift a present host cellular immune response to an antigen away from a Th2 phenotype and into a Th1 phenotype. To this end, ISS-ODN are delivered by any route through which antigen-sensitized host tissues will be contacted with the ISS-ODN. ISS-ODN administered in this fashion boost both humoral (antibody) and cellular (Th1 type) immune responses of the host. Unlike canonical immunotherapy immunity is stimulated by this method of the invention even when no additional antigen is introduced into the host. Thus, use of the method to boost the immune responsiveness of a host to subsequent challenge by a sensitizing antigen without immunization avoids the risk of immunization-induced anaphylaxis, suppresses IgE production in response to the antigen challenge and eliminates the need to identify the sensitizing antigen for use in immunization. An especially advantageous use for this aspect of the invention is treatment of localized allergic responses in target tissues where the allergens enter the body, such as the skin and mucosa.

[0010] Suppression of the Th2 phenotype according to the invention is also a useful adjunct to canonical immunotherapy to reduce antigen-stimulated IL-4 and IL-5 production. Thus, the invention encompasses delivery of ISS-ODN to a host to suppress the Th2 phenotype associated with conventional antigen immunization (e.g., for vaccination or allergy immunotherapy).

[0011] The shift to a Th1 phenotype achieved according to the invention is accompanied by increased secretion of IFN .alpha., .beta., and .gamma., as well as IL-12 and IL-18. Each of these cytokines enhance the host's immune defenses against intracellular pathogens, such as viruses. Thus, the invention encompasses delivery of ISS-ODN to a host to combat pathogenic infection.

[0012] Angiogenesis is also enhanced in the Th1 phenotype (ostensibly through stimulation by IL-12). Thus, the invention encompasses delivery of ISS-ODN to a host to stimulate therapeutic angiogenesis to treat conditions in which localized blood flow plays a significant etiological role, such as in diabetic retinopathy.

[0013] Pharmaceutically acceptable compositions of ISS-ODN are provided for use in practicing the methods of the invention. The ISS-ODN of the invention include DNA or RNA oligonucleotides which are enriched with CpG dinucleotides, including those which are comprised of the primary structure 5'-

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Purine-Purine-[C]-[G]-Pyrimidine-Pyrimidine-3'.

[0014] Where appropriate to the contemplated course of therapy, the ISS-ODN may be administered with other anti-inflammatory or immunotherapeutic agents. Thus, a particularly useful composition for use in practicing the method of the invention is one in which an anti-inflammatory agent (e.g., a glucocorticoid) or immunotherapeutic agent (e.g., an antigen, cytokine or adjuvant) is mixed with, or conjugated to, an ISS-ODN.

[0015] The ISS-ODN can also be provided in the form of a kit comprising ISS-ODN and any additional medicaments, as well as a device for delivery of the ISS-ODN to a host tissue and reagents for determining the biological effect of the ISS-ODN on the treated host.

BRIEF DESCRIPTION OF DRAWINGS

[0016] FIG. 1 is a chart which summarizes aspects of the mammalian immune system.

[0017] FIG. 2 is a graph of data which confirm a shift from a Th2 to a Th1 phenotype (as measured by IgG2A production) in mice treated with an ISS-ODN 3 days before antigen challenge.

[0018] FIGS. 3a and 3b are graphs of data which confirm the induction of a Th2 phenotype (as measured by IgG1 production) in mice treated with a mutant, inactive ISS-ODN 3 days before antigen challenge.

[0019] FIG. 4 is a graph of data which confirm Th1-associated suppression of antigen-specific IgE in antigen-sensitized, ISS-ODN (pCMV-LacZ, a plasmid containing two copies of the DY1018 ISS-ODN) treated mice as compared to antigen-sensitized (control) mice.

[0020] FIG. 5 is a graph of data which confirm suppression of IL-4 secretion by ISS-ODN as compared to a control.

[0021] FIG. 6 is a graph of data which confirm suppression of IL-5 secretion by ISS-ODN as compared to a control.

[0022] FIG. 7 is a graph of data which confirm suppression of IL-10 secretion by ISS-ODN as compared to a control.

[0023] FIG. 8 is a graph of data which confirm stimulation of INF-.gamma. secretion by ISS-ODN as compared to a control.

[0024] FIG. 9 is a graph of data demonstrating an ISS-ODN mediated shift to a Th1 phenotype (as indicated by IFN.gamma. levels) in animals treated with ISS-ODN before antigen challenge (asterisked bars) or after antigen challenge.

[0025] FIG. 10 is a graph of data demonstrating an ISS-ODN mediated boost in immune responsiveness (as indicated by increases in CD4+ lymphocyte proliferation) in animals treated with ISS-ODN before antigen challenge (asterisked bars) or after antigen challenge.

DETAILED DESCRIPTION OF THE INVENTION

[0026] A. Anti-Inflammatory and Immunotherapeutic Methods of the Invention

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[0027] 1. Therapeutic Effects of the Methods of the Invention

[0028] The main therapeutic goals which may be achieved through practice of the methods of the invention are treatment of inflammation and boosting of host immune responsiveness with a Th1 phenotype against a sensitizing antigen. Both goals are achieved by delivering ISS-ODN to an antigen-sensitized host; i.e., a mammal whose immune system has been primed to respond to challenge by a sensitizing antigen. For purposes of this disclosure, "sensitizing antigen" refers to an exogenous, immunogenic protein, peptide, glycoprotein, lipid or polysaccharide. For reference, a chart summarizing aspects of mammal antigen immunity is appended as FIG. 1.

[0029] The anti-inflammatory method of the invention is useful in suppressing the onset of, and in reducing, acute granulocyte-mediated inflammation in an antigen-sensitized host. Specifically, treatment of an antigen-sensitized (primed) host before subsequent antigen challenge suppresses antigen-stimulated infiltration of host tissue by granulocytes (especially, eosinophils and basophils). Similarly, treatment of an antigen-sensitized host on or after antigen challenge reduces antigen-stimulated infiltration of host tissue by granulocytes. Advantageously, the anti-inflammatory impact of ISS-ODN delivered according to the invention is rapid, taking effect even before the ISS-ODN would be expected to impact the host's immune responsiveness to the sensitizing antigen. The invention therefore provides the host with fairly immediate protection against tissue damage from granulocyte-mediated inflammation.

[0030] For example, as shown by the data in Example II, antigen-sensitized animal models of allergic asthma treated with ISS-ODN without concurrent antigen challenge experienced as much as a 90% reduction of eosinophil infiltration into respiratory tissue as compared to control animals and animals treated only with an inactive ISS-ODN mutant. Significantly, reduction of eosinophil infiltration in previously challenged mice, or suppression of eosinophil infiltration in primed, unchallenged mice, was obtained within as little as 24 hours of delivery of the ISS-ODN. The effect of the ISS-ODN on eosinophil infiltration is therefore independent of the later-developing host immune response to the sensitizing antigen. Being antigen independent, the ISS-ODN can be utilized as inflammation suppressors before antigen challenge or during a period when the risk of antigen challenge is present (e.g., during an allergy season). Importantly, as shown in Examples IV and VI, ISS-ODN can be used according to the invention to prevent inflammation or an immune response on subsequent antigen challenge in an antigen-primed host as well as to reduce inflammation or other antigen-stimulated immune responses after antigen challenge.

[0031] Although the invention is not limited to any mechanism of action, it is probable that the anti-inflammatory activity of ISS-ODN is at least in part a consequence of IL-5 suppression. However, suppression of granulocyte accumulation in host tissue is achieved more rapidly (within 24 hours) than immune activation of cytokine-secreting lymphocytes would be expected to occur. It is therefore also possible that ISS-ODN administered according to the invention physically interfere with granulocyte adhesion to endothelial, perhaps by blocking VCAM-1 endothelial receptors, their eosinophilic ligand (VLA-4) or by lysing granulocytes. Whatever the mechanism, ISS-ODN suppression of granulocyte accumulation according to the invention appears to be independent of ISS-ODN stimulation of the host immune system.

[0032] The immunotherapeutic method of the invention produces a vaccination-like immune response to challenge by a sensitizing antigen without concurrent exposure of the host to the antigen. Immune stimulation achieved through practice of the invention is comparable to the immune stimulation which occurs on vaccination of a host with a sensitizing antigen. Thus, the methods of the invention provides means to immunize a host against a sensitizing antigen without deliberate antigen challenge.

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[0033] Advantageously, the immune response stimulated according to the invention differs from an immunization response in that the latter develops in a Th2 phenotype while the former develops in a Th1 phenotype. In this regard, it is helpful to recall that CD4+ lymphocytes generally fall into one of two distinct subsets; i.e., the Th1 and Th2 cells. Th1 cells principally secrete IL-2, IFN.gamma. and TNF.beta. (the latter two of which mediate macrophage activation and delayed type hypersensitivity) while Th2 cells principally secrete IL-4 (which stimulates production of IgE antibodies), IL-5 (which stimulates granulocyte infiltration of tissue), IL-6 and IL-10. These CD4+ subsets exert a negative influence on one another; i.e., secretion of Th1 lymphokines inhibits secretion of Th2 lymphokines and vice versa.

[0034] Factors believed to favor Th1 activation resemble those induced by viral infection and include intracellular pathogens, exposure to IFN-.beta., IFN-.alpha., IFN.gamma., IL-12 and IL-18 and exposure to low doses of antigen. Th1 type immune responses also predominate in autoimmune disease. Factors believed to favor Th2 activation include exposure to IL-4 and IL-10, APC activity on the part of .beta.-lymphocytes and high doses of antigen. Active Th1 (IFN.gamma.) cells enhance cellular immunity and are therefore of particular value in responding to intracellular infections, while active Th2 cells enhance antibody production and are therefore of value in responding to extracellular infections (at the risk of anaphylactic events associated with IL-4 stimulated induction of IgE antibody production). Thus, the ability to shift host immune responses from the Th1 to the Th2 repertoire and vice versa has substantial clinical significance for controlling host immunity against antigen challenge (e.g., in infectious and allergic conditions).

[0035] To that end, the methods of the invention shift the host immune response to a sensitizing antigen toward a Th1 phenotype (Example IV). Consequently, antigen-stimulated/Th2 associated IL-4, IL-5 and IL-10 secretion (Example VI), IL-5 stimulated granulocyte infiltration of antigen-sensitized tissue (Examples II and III) and IL-4 stimulated production of IgE (Example V) are suppressed, thereby reducing the host's risk of prolonged allergic inflammation and minimizing the risk of antigen-induced anaphylaxis. Although the invention is not limited to any particular mechanism of action, it is conceivable that ISS-ODN facilitate uptake of exogenous antigen by antigen presenting cells for presentation through host MHC Class I processing pathways. Whatever the mechanism of action, use of ISS-ODN to boost the host's immune responsiveness to a sensitizing antigen and shift the immune response toward a Th1 phenotype avoids the risk of immunization-induced anaphylaxis, suppresses IgE production in response to a sensitizing antigen and eliminates the need to identify the sensitizing antigen for use in immunization.

[0036] With reference to the invention, ISS-ODN mediated "reduction of inflammation" (in a primed, antigen-challenged host), "prevention of inflammation" (in a primed host before antigen challenge) and "boosting of immune responsiveness in a Th1 phenotype" in an ISS-ODN treated host are evidenced by any of the following events:

[0037] (1) a reduction in levels of IL-4 measured before and after antigen-challenge; or detection of lower (or even absent) levels of IL-4 in a treated host as compared to an antigen-primed, or primed and challenged, control;

[0038] (2) an increase in levels of IL-12, IL-18 and/or IFN (.alpha., .beta. or .gamma.) before and after antigen challenge; or detection of higher levels of IL-12, IL-18 and/or IFN (.alpha., .beta. or .gamma.) in an ISS-ODN treated host as compared to an antigen-primed or, primed and challenged, control;

[0039] (3) IgG2a antibody production in a treated host; or

[0040] (4) a reduction in levels of antigen-specific IgE as measured before and after antigen challenge;

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or detection of lower (or even absent) levels of antigen-specific IgE in an ISS-ODN treated host as compared to an antigen-primed, or primed and challenged, control.

[0041] Also, with respect to reduction and prevention of inflammation in particular, an especially meaningful indicia of the efficacy of the inventive method in a treated host is:

[0042] (5) a reduction in granulocyte counts (e.g., of eosinophils or basophils, depending on which cell type is most involved in the condition affecting the host) in inflammatory infiltrate of an affected host tissue as measured in an antigen-challenged host before and after ISSODN administration, or detection of lower (or even absent) levels of eosinophil or basophil counts in a treated host as compared to an antigen-primed, or primed and challenged, control.

[0043] Exemplary methods for determining such values are described further in the Examples.

[0044] 2. Methods and Routes for Administration of ISS-ODN to a Host

[0045] The ISS-ODN of the invention are administered to a host using any available method and route suitable for drug delivery, including ex vivo methods (e.g., delivery of cells incubated or transfected with an ISS-ODN) as well as systemic or localized routes. However, those of ordinary skill in the art will appreciate that methods and localized routes which direct the ISS-ODN into antigen-sensitized tissue will be preferred in most circumstances to systemic routes of administration, both for immediacy of therapeutic effect and avoidance of oligonucleotide degradation in vivo.

[0046] The entrance point for many exogenous antigens into a host is through the skin or mucosa. Thus, delivery methods and routes which target the skin (e.g., for cutaneous and subcutaneous conditions) or mucosa (e.g., for respiratory, ocular, lingual or genital conditions) will be especially useful. Those of ordinary skill in the clinical arts will be familiar with, or can readily ascertain, means for drug delivery into skin and mucosa. For review, however, exemplary methods and routes of drug delivery useful in the invention are briefly discussed below.

[0047] Intranasal administration means are particularly useful in addressing respiratory inflammation, particularly inflammation mediated by antigens transmitted from the nasal passages into the trachea or bronchioli. Such means include inhalation of aerosol suspensions or insufflation of the polynucleotide compositions of the invention. Nebulizer devices suitable for delivery of polynucleotide compositions to the nasal mucosa, trachea and bronchioli are well-known in the art and will therefore not be described in detail here. For general review in regard to intranasal drug delivery, those of ordinary skill in the art may wish to consult Chien, Novel Drug Delivery Systems, Ch. 5 (Marcel Dekker, 1992).

[0048] Dermal routes of administration, as well as subcutaneous injections, are useful in addressing allergic reactions and inflammation in the skin. Examples of means for delivering drugs to the skin are topical application of a suitable pharmaceutical preparation, transdermal transmission, injection and epidermal administration.

[0049] For transdermal transmission, absorption promoters or iontophoresis are suitable methods. For review regarding such methods, those of ordinary skill in the art may wish to consult Chien, supra at Ch. 7. Iontophoretic transmission may be accomplished using commercially available "patches" which deliver their product continuously via electric pulses through unbroken skin for periods of several days or more. Use of this method allows for controlled transmission of pharmaceutical compositions in relatively great concentrations, permits infusion of combination drugs and allows for contemporaneous use of an absorption promoter.

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[0050] An exemplary patch product for use in this method is the LECTRO PATCH trademarked product of General Medical Company of Los Angeles, Calif. This product electronically maintains reservoir electrodes at neutral pH and can be adapted to provide dosages of differing concentrations, to dose continuously and/or to dose periodically. Preparation and use of the patch should be performed according to the manufacturer's printed instructions which accompany the LECTRO PATCH product; those instructions are incorporated herein by this reference.

[0051] Epidermal administration essentially involves mechanically or chemically irritating the outermost layer of the epidermis sufficiently to provoke an immune response to the irritant. An exemplary device for use in epidermal administration employs a multiplicity of very narrow diameter, short tynes which can be used to scratch ISS-ODN coated onto the tynes into the skin. The device included in the MONO-VACC old tuberculin test manufactured by Pasteur Merieux of Lyon, France is suitable for use in epidermal administration of ISS-ODN. Use of the device is according to the manufacturer's written instructions included with the device product; these instructions regarding use and administration are incorporated herein by this reference to illustrate conventional use of the device. Similar devices which may also be used in this embodiment are those which are currently used to perform allergy tests.

[0052] Ophthalmic administration (e.g., for treatment of allergic conjunctivitis) involves invasive or topical application of a pharmaceutical preparation to the eye. Eye drops, topical cremes and injectable liquids are all examples of suitable milieus for delivering drugs to the eye.

[0053] Systemic administration involves invasive or systemically absorbed topical administration of pharmaceutical preparations. Topical applications as well as intravenous and intramuscular injections are examples of common means for systemic administration of drugs.

[0054] 3. Dosing Parameters for ISS-ODN

[0055] A particular advantage of the ISS-ODN of the invention is their capacity to exert anti-inflammatory and immunotherapeutic activity even at relatively minute dosages. Although the dosage used will vary depending on the clinical goals to be achieved, a suitable dosage range is one which provides up to about 1-1000 .mu.g of ISS-ODN/ml of carrier in a single dosage. In view of the teaching provided by this disclosure, those of ordinary skill in the clinical arts will be familiar with, or can readily ascertain, suitable parameters for administration of ISS-ODN according to the invention.

[0056] In this respect, it should be noted that the anti-inflammatory and immunotherapeutic activity of ISS-ODN in the invention is essentially dose-dependent. Therefore, to increase ISS-ODN potency by a magnitude of two, each single dose is doubled in concentration. Clinically, it may be advisable to administer the ISS-ODN in a low dosage (e.g., about 1 .mu.g/ml to about 50 .mu.g/ml), then increase the dosage as needed to achieve the desired therapeutic goal. Alternatively, a target dosage of ISS-ODN can be considered to be about 1-10 .mu.M in a sample of host blood drawn within the first 24-48 hours after administration of ISS-ODN. Based on current studies, ISS-ODN are believed to have little or no toxicity at these dosage levels.

[0057] B. ISS-ODN Anti-Inflammatory Compositions

[0058] 1. ISS-ODN Structure

[0059] Functionally, ISS-ODN enhance the cellular and humoral immune responses in a host, particularly lymphocyte proliferation and the release of cytokines (including IFN) by host monocytes and natural killer (NK) cells. Immunostimulation by synthetic ISS-ODN in vivo occurs by contacting host lymphocytes with, for example, ISS-ODN oligonucleotides, ISS-ODN oligonucleotide-conjugates

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and ISS-containing recombinant expression vectors (data regarding the activity of ISS-ODN conjugates and ISS-ODN vectors are set forth in co-pending, commonly assigned U.S. patent applications Serial No., 60/028,118 and Ser. No. 08/593,554; data from which is incorporated herein by reference to demonstrate ISS-ODN immunostimulatory activity in vivo). Thus, while native microbial ISS-ODN stimulate the host immune system to respond to infection, synthetic analogs of these ISS-ODN are useful therapeutically to modulate the host immune response not only to microbial antigens, but also to tumor antigens, allergens and other substances. Structurally, ISS-ODN are non-coding oligonucleotides 6 mer or greater in length which may include at least one unmethylated CpG motif. The relative position of each CpG sequence in ISS-ODN with immunostimulatory activity in certain mammalian species (e.g., rodents) is 5'-CG-3' (i.e., the C is in the 5' position with respect to the G in the 3' position). Many known ISS-ODN flank the CpG motif with at least two purine nucleotides (e.g., GA or AA) and at least two pyrimidine nucleotides (5'-Purine-Purine[C]-[G]-Pyrimidine-Pyri- midine-3'). CpG motif-containing ISS-ODN are believed to stimulate B lymphocyte proliferation (see, e.g., Krieg, et al., Nature, 374:546-549, 1995).

[0060] The core hexamer structure of the foregoing ISS-ODN may be flanked upstream and/or downstream by any number or composition of nucleotides or nucleosides. However, ISS-ODN are at least 6 mer in length, and preferably are between 6 and 200 mer in length, to enhance uptake of the ISS-ODN into target tissues. Those of ordinary skill in the art will be familiar with, or can readily identify, reported nucleotide sequences of known ISS-ODN. For ease of reference in this regard, the following sources are especially helpful:

[0061] Yamamoto, et al., Microbiol. Immunol., 36:983 (1992)

[0062] Ballas, et al., J. Immunol., 157:1840 (1996)

[0063] Klinman, et al., J. Immunol., 158:3635 (1997)

[0064] Sato, et al., Science, 273:352 (1996)

[0065] Each of these articles are incorporated herein by reference for the purpose of illustrating the level of knowledge in the art concerning the nucleotide composition of ISS-ODN.

[0066] In particular, ISS-ODN useful in the invention include those which have the following hexameric nucleotide sequences:

[0067] 1. ISS-ODN having "CpG" dinucleotides; and,

[0068] 2. Inosine and/or uracil substitutions for nucleotides in the foregoing hexamer sequences for use as RNA ISS-ODN.

[0069] For example, DNA based ISS-ODN useful in the invention include those which have the following hexameric nucleotide sequences:

[0070] AACGTT, AGCGTC, GACGTT, GGCGTT, AACGTC, AGCGTC, GACGTC, GGCGTC, AACGCC, AGCGCC, GACGCC, GGCGCC, AGCGCT, GACGCT, GGCGCT, TTCGAA, GGCGTT and AACGCC (respectively, SEQ.ID.Nos. 1-18).

[0071] ISS-ODN may be single-stranded or double-stranded DNA, single or double-stranded RNA and/or oligonucleosides. The ISS-ODN may or may not include palindromic regions. If present, a palindrome may extend only to a CpG motif, if present, in the core hexamer sequence, or may

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encompass more of the hexamer sequence as well as flanking nucleotide sequences.

[0072] The nucleotide bases of the ISS-ODN which flank the CpG motif of the core hexamer and/or make up the flanking nucleotide sequences may be any known naturally occurring bases or synthetic nonnatural bases (e.g., TCAG or, in RNA, UACGI). Oligonucleosides may be incorporated into the internal region and/or termini of the ISS-ODN using conventional techniques for use as attachment points for other compounds (e.g., peptides). The base(s), sugar moiety, phosphate groups and termini of the ISS-ODN may also be modified in any manner known to those of ordinary skill in the art to construct an ISS-ODN having properties desired in addition to the described activity of the ISS-ODN. For example, sugar moieties may be attached to nucleotide bases of ISS-ODN in any steric configuration.

[0073] In addition, backbone phosphate group modifications (e.g., methylphosphonate, phosphorothioate, phosphoroamidate and phosphorodithioate internucleotide linkages) can confer anti-microbial activity on the ISS-ODN and enhance their stability in vivo, making them particularly useful in therapeutic applications. A particularly useful phosphate group modification is the conversion to the phosphorothioate or phosphorodithioate forms of the ISS-ODN oligonucleotides. In addition to their potentially anti-microbial properties, phosphorothioates and phosphorodithioates are more resistant to degradation in vivo than their unmodified oligonucleotide counterparts, making the ISS-ODN of the invention more available to the host.

[0074] 2. Synthesis of, and Screening for, ISS-ODN

[0075] ISS-ODN can be synthesized using techniques and nucleic acid synthesis equipment which are well-known in the art. For reference in this regard, see, e.g., Ausubel, et al., Current Protocols in Molecular Biology, Chs. 2 and 4 (Wiley Interscience, 1989); Maniatis, et al., Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Lab., New York, 1982); U.S. Pat. No. 4,458,066 and U.S. Pat. No. 4,650,675. These references are incorporated herein by reference for the sole purpose of demonstrating knowledge in the art concerning production of synthetic oligonucleotides. Because the ISS-ODN is non-coding, there is no concern about maintaining an open reading frame during synthesis.

[0076] ISS-ODN may be incorporated into a delivery vector, such as a plasmid, cosmid, virus or retrovirus, which may in turn code for therapeutically beneficial polypeptides, such as cytokines, hormones and antigens. Incorporation of ISS-ODN into such a vector does not adversely affect their activity.

[0077] Alternatively, ISS-ODN may be isolated from microbial species (especially mycobacteria) using techniques well-known in the art, such as nucleic acid hybridization. Preferably, such isolated ISS-ODN will be purified to a substantially pure state; i.e., to be free of endogenous contaminants, such as lipopolysaccharides. ISS-ODN isolated as part of a larger polynucleotide can be reduced to the desired length by techniques well known in the art, such as by endonuclease digestion. Those of ordinary skill in the art will be familiar with, or can readily ascertain, techniques suitable for isolation, purification and digestion of polynucleotides to obtain ISS-ODN of potential use in the invention.

[0078] Confirmation that a particular oligonucleotide has the properties of an ISS-ODN useful in the invention can be obtained by evaluating whether the ISS-ODN affects cytokine secretion and IgG antibody isotype production as described in Section A.I, above. Details of in vitro techniques useful in making such an evaluation are given in the Examples; those of ordinary skill in the art will also know of, or can readily ascertain, other methods for measuring cytokine secretion and antibody production along the parameters taught herein.

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[0079] The techniques for making phosphate group modifications to oligonucleotides are known in the art and do not require detailed explanation. For review of one such useful technique, the an intermediate phosphate triester for the target oligonucleotide product is prepared and oxidized to the naturally occurring phosphate triester with aqueous iodine or with other agents, such as anhydrous amines. The resulting oligonucleotide phosphoramidates can be treated with sulfur to yield phophorothioates. The same general technique (excepting the sulfur treatment step) can be applied to yield methylphosphoamidites from methylphosphonates. For more details concerning phosphate group modification techniques, those of ordinary skill in the art may wish to consult U.S. Pat. Nos. 4,425,732; 4,458,066; 5,218,103 and 5,453,496, as well as Tetrahedron Lett. at 21:4149 (1995), 7:5575 (1986), 25:1437 (1984) and Journal Am. Chem Soc., 93:6657 (1987), the disclosures of which are incorporated herein for the sole purpose of illustrating the standard level of knowledge in the art concerning preparation of these compounds.

[0080] A colloidal dispersion system may be used for targeted delivery of the ISS-ODN to an inflamed tissue. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. The preferred colloidal system of this invention is a liposome.

[0081] Liposomes are artificial membrane vesicles which are useful as delivery vehicles in vitro and in vivo. It has been shown that large unilamellar vesicles (LUV), which range in size from 0.2-4.0 .mu.m can encapsulate a substantial percentage of an aqueous buffer containing large macromolecules. RNA, DNA and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley, et al., Trends Biochem. Sci., 6:77, 1981). In addition to mammalian cells, liposomes have been used for delivery of polynucleotides in plant, yeast and bacterial cells. In order for a liposome to be an efficient gene transfer vehicle, the following characteristics should be present: (1) encapsulation of the genes encoding the antisense polynucleotides at high efficiency while not compromising their biological activity; (2) preferential and substantial binding to a target cell in comparison to non-target cells; (3) delivery of the aqueous contents of the vesicle to the target cell cytoplasm at high efficiency; and (4) accurate and effective expression of genetic information (Mannino, et al., Biotechniques, 6:682, 1988).

[0082] The composition of the liposome is usually a combination of phospholipids, particularly high-phasetransition-temperature phospholipids, usually in combination with steroids, especially cholesterol. Other phospholipids or other lipids may also be used. The physical characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations.

[0083] Examples of lipids useful in liposome production include phosphatidyl compounds, such as phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebrosides, and gangliosides. Particularly useful are iacylphosphatidylglycerols, where the lipid moiety contains from 14-18 carbon atoms, particularly from 16-18 carbon atoms, and is saturated. Illustrative phospholipids include egg phosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine.

[0084] The targeting of liposomes can be classified based on anatomical and mechanistic factors. Anatomical classification is based on the level of selectivity, for example, organ-specific, cell-specific, and organelle-specific. Mechanistic targeting can be distinguished based upon whether it is passive or active. Passive targeting utilizes the natural tendency of liposomes to distribute to cells of the reticulo-endothelial system (RES) in organs which contain sinusoidal capillaries. Active targeting, on the other hand, involves alteration of the liposome by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein, or by changing the composition or size of the liposome in order to achieve targeting to organs and cell types other than the naturally occurring sites of

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localization.

[0085] The surface of the targeted delivery system may be modified in a variety of ways. In the case of a liposomal targeted delivery system, lipid groups can be incorporated into the lipid bilayer of the liposome in order to maintain the targeting ligand in stable association with the liposomal bilayer. Various well known linking groups can be used for joining the lipid chains to the targeting ligand (see, e.g., Yanagawa, et al., Nuc. Acids Symp. Ser., 19:189 (1988), Grabarek, et al., Anal. Biochem., 185:131 (1990); Staros, et al., Anal. Biochem., 156:220 (1986) and Boujrad, et al., Proc. Natl. Acad. Sci USA, 90:5728 (1993), the disclosures of which are incorporated herein by reference solely to illustrate the standard level of knowledge in the art concerning conjugation of oligonucleotides to lipids). Targeted delivery of ISS-ODN can also be achieved by conjugation of the ISS-ODN to a the surface of viral and non-viral recombinant expression vectors, to an antigen or other ligand, to a monoclonal antibody or to any molecule which has the desired binding specificity.

[0086] Examples of other useful conjugate partners include any immunogenic antigen (including allergens, live and attenuated viral particles and tumor antigens), targeting peptides (such as receptor ligands, antibodies and antibody fragments, hormones and enzymes), non-peptidic antigens (coupled via a peptide linkage, such as lipids, polysaccharides, glycoproteins, gangliosides and the like) and cytokines (including interleukins, interferons, erythropoietin, tumor necrosis factor and colony stimulating factors). Such conjugate partners can be prepared according to conventional techniques (e.g., peptide synthesis) and many are commercially available.

[0087] Those of ordinary skill in the art will also be familiar with, or can readily determine, methods useful in preparing oligonucleotide-peptide conjugates. Conjugation can be accomplished at either termini of the ISS-ODN or at a suitably modified base in an internal position (e.g., a cytosine or uracil). For reference, methods for conjugating oligonucleotides to proteins and to oligosaccharide moieties of Ig are known (see, e.g., O'Shannessy, et al., J. Applied Biochem., 7:347 (1985), the disclosure of which is incorporated herein by reference solely to illustrate the standard level of knowledge in the art concerning oligonucleotide conjugation). Another useful reference is Kessler: "Nonradioactive Labeling Methods for Nucleic Acids", in Kricka (ed.), Nonisotopic DNA Probe Techniques (Acad.Press, 1992)).

[0088] Briefly, examples of known, suitable conjugation methods include: conjugation through 3' attachment via solid support chemistry see, e.g., Haralambidis, et al., Nuc. Acids Res., 18:493 (1990) and Haralambidis, et al., Nuc. Acids Res., 18:501 (1990) [solid support synthesis of peptide partner]; Zuckermann, et al., Nuc. Acids Res., 15:5305 (1987), Corey, et al., Science, 238:1401 (1987) and Nelson, et al., Nuc. Acids Res., 17:1781 (1989) [solid support synthesis of oligonucleotide partner]).

[0089] Amino-amino group linkages may be performed as described in Benoit. et al., Neuromethods, 6:43 (1987), while thiol-carboxyl group linkages may be performed as described in Sinah, et al., Oligonucleotide Analogues: A Practical Approach (IRL Press, 1991). In these latter methods, the oligonucleotide partner is synthesized on a solid support and a linking group comprising a Protected amine, thiol or carboxyl group opposite a phosphoramidite is covalently attached to the 5'-hydroxyl (see, e.g., U.S. Pat. Nos. 4,849,513; 5,015,733; 5,118,800 and 5,118,802).

[0090] Linkage of the oligonucleotide partner to a peptide may also be made via incorporation of a linker arm (e.g., amine or carboxyl group) to a modified cytosine or uracil base (see, e.g., Ruth, 4th Annual Congress for Recombinant DNA Research at 123). Affinity linkages (e.g., biotin-streptavidin) may also be used (see, e.g., Roget, et al, Nuc. Acids Res., 17:7643 (1989)).

[0091] Methods for linking oligonucleotides to lipids are also known and include synthesis of oligophospholipid conjugates see, e.g., Yanagawa, et al., Nuc. Acids Symp. Ser., 19:189 (1988)),

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synthesis of oligo-fatty acids conjugates (see, e.g., Grabarek, et al, Anal. Biochem., 185:131 (1990)) and oligo-sterol conjugates (see, e.g., Boujrad, et al., Proc. Natl. Acad. Sci USA, 90:5728 (1993)).

[0092] Each of the foregoing references is incorporated herein by reference for the sole purpose of illustrating the level of knowledge and skill in the art with respect to oligonucleotide conjugation methods.

[0093] Co-administration of a peptide drug with an ISS-ODN according to the invention may also be achieved by incorporating the ISS-ODN in cis or in trans into a recombinant expression vector (plasmid, cosmid, virus or retrovirus) which codes for any therapeutically beneficial protein deliverable by a recombinant expression vector. If incorporation of an ISS-ODN into an expression vector for use in practicing the invention is desired, such incorporation may be accomplished using conventional techniques which do not require detailed explanation to one of ordinary skill in the art. For review, however, those of ordinary skill may wish to consult Ausubel, Current Protocols in Molecular Biology, supra.

[0094] Briefly, construction of recombinant expression vectors (including those which do not code for any protein and are used as carriers for ISS-ODN) employs standard ligation techniques. For analysis to confirm correct sequences in vectors constructed, the ligation mixtures may be used to transform a host cell and successful transformants selected by antibiotic resistance where appropriate. Vectors from the transformants are prepared, analyzed by restriction and/or sequenced by, for example, the method of Messing, et al., (Nucleic Acids Res., 9-309, 1981), the method of Maxam, et al., (Methods in Enzymology, 65:499,1980), or other suitable methods which will be known to those skilled in the art. Size separation of cleaved fragments is performed using conventional gel electrophoresis as described, for example, by Maniatis, et al., (Molecular Cloning, pp. 133-134, 1982).

[0095] Host cells may be transformed with expression vectors and cultured in conventional nutrient media modified as is appropriate for inducing promoters, selecting transformants or amplifying genes. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

[0096] If a recombinant expression vector is utilized as a carrier for the ISS-ODN of the invention, plasmids and cosmids are particularly preferred for their lack of pathogenicity. However, plasmids and cosmids are subject to degradation in vivo more quickly than viruses and therefore may not deliver an adequate dosage of ISS-ODN to substantially inhibit ISS-ODN immunostimulatory activity exerted by a systemically administered gene therapy vector. Of the viral vector alternatives, adenoassociated viruses would possess the advantage of low pathogenicity. The relatively low capacity of adeno-associated viruses for insertion of foreign genes would pose no problem in this context due to the relatively small size in which ISS-ODN of the invention can be synthesized.

[0097] Other viral vectors that can be utilized in the invention include adenovirus, adeno-associated virus, herpes virus, vaccinia or an RNA virus such as a retrovirus. Retroviral vectors are preferably derivatives of a murine, avian or human HIV retrovirus. Examples of retroviral vectors in which a single foreign gene can be inserted include, but are not limited to: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV). A number of additional retroviral vectors can incorporate multiple genes. All of these vectors can transfer or incorporate a gene for a selectable marker so that transduced cells can be identified and generated.

[0098] Since recombinant retroviruses are defective, they require assistance in order to produce infectious vector particles. This assistance can be provided, for example, by using helper cell lines that

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contain plasmids encoding all of the structural genes of the retrovirus under the control of regulatory sequences within the LTR. These plasmids are missing a nucleotide sequence that enables the packaging mechanism to recognize an RNA transcript for encapsidation. Helper cell lines that have deletions of the packaging signal include, but are not limited to, T2, PA317 and PA12, for example. These cell lines produce empty virions, since no genome is packaged. If a retroviral vector is introduced into such helper cells in which the packaging signal is intact, but the structural genes are replaced by other genes of interest, the vector can be packaged and vector virion can be produced.

[0099] By inserting one or more sequences of interest into the viral vector, along with another gene which encodes the ligand for a receptor on a specific target cell, for example, the vector can be rendered target specific. Retroviral vectors can be made target specific by inserting, for example, a polynucleotide encoding a sugar, a glycolipid, or a protein. Preferred targeting is accomplished by using an antibody to target the retroviral vector. Those of skill in the art will know of, or can readily ascertain without undue experimentation, specific polynucleotide sequences which can be inserted into the retroviral genome to allow target specific delivery of the retroviral vector containing ISS-ODN.

[0100] C. Pharmaceutical Compositions of ISS-ODN

[0101] If to be delivered without use of a vector or other delivery system, ISS-ODN will be prepared in a pharmaceutically acceptable composition. Pharmaceutically acceptable carriers preferred for use with the ISS-ODN of the invention may include sterile aqueous of non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, antioxidants, chelating agents, and inert gases and the like. A composition of ISS-ODN may also be lyophilized using means well known in the art, for subsequent reconstitution and use according to the invention.

[0102] Absorption promoters, detergents and chemical irritants (e.g., keratinolytic agents) can enhance transmission of an ISS-ODN composition into a target tissue. For reference concerning general principles regarding absorption promoters and detergents which have been used with success in mucosal delivery of organic and peptide-based drugs, see Chien, Novel Drug Delivery Systems, Ch. 4 (Marcel Dekker, 1992).

[0103] Examples of suitable nasal absorption promoters in particular are set forth at Chien, supra at Ch. 5, Tables 2 and 3; milder agents are preferred. Suitable agents for use in the method of this invention for mucosal/nasal delivery are also described in Chang, et al., Nasal Drug Delivery, "Treatise on Controlled Drug Delivery", Ch. 9 and Table 3-4B thereof, (Marcel Dekker, 1992). Suitable agents which are known to enhance absorption of drugs through skin are described in Sloan, Use of Solubility Parameters from Regular Solution Theory to Describe Partitioning-Driven Processes, Ch. 5, "Prodrugs: Topical and Ocular Drug Delivery" (Marcel Dekker, 1992), and at places elsewhere in the text. All of these references are incorporated herein for the sole purpose of illustrating the level of knowledge and skill in the art concerning drug delivery techniques.

[0104] D. Kits for Use in Practicing the Methods of the Invention

[0105] For use in the methods described above, kits are also provided by the invention. Such kits may include any or all of the following: ISS-ODN (conjugated or unconjugated); a pharmaceutically

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acceptable carrier (may be pre-mixed with the ISS-ODN) or suspension base for reconstituting lyophilized ISS-ODN; additional medicaments; a sterile vial for each ISS-ODN and additional medicament, or a single vial for mixtures thereof; device(s) for use in delivering ISS-ODN to a host; assay reagents for detecting indicia that the anti-inflammatory and/or immunostimulatory effects sought have been achieved in treated animals and a suitable assay device.

[0106] Examples illustrating the practice of the invention are set forth below. The examples are for purposes of reference only and should not be construed to limit the invention, which is defined by the appended claims. All abbreviations and terms used in the examples have their expected and ordinary meaning unless otherwise specified.

EXAMPLE I

Murine Model for the Airway Hyperreactivity of Allergic Asthma

[0107] Sensitizing-antigen challenged mice of different strains model the airway hyperreactivity seen in allergic asthma. Suitable murine strains for use in modeling the disease include Balb/c mice (which are biased genetically toward the Th2 phenotype and produce enhanced concentrations of IL-4 and IL-5 in response to antigen challenge to CD4+ lymphocytes), C57BL/6 mice (which are IL-5 deficient, for detailed study of IL-5 induced tissue damage in asthma) and W/W.sup.v mice (which are mast cell deficient, for detailed study of mast cell activation in asthma).

[0108] Disease modeling mice are conveniently prepared by intraperitoneal or subcutaneous injection of a model sensitizing antigen, ovalbumin ("OVA") in carrier (e.g., sterile saline or a carrier with adjuvant, such as alum), followed by antigen challenge with aerosolized antigen. For example, mice may be immunized with 25 .mu.g OVA by subcutaneous injection (with or without adjuvant) weekly for 4-6 weeks, then challenged with 2 or 3 weekly aerosolizations of OVA at a concentration of 50 mg/ml in phosphate buffered saline (PBS) delivered in 20 minute intervals or at a concentration of 10 mg/ml 0.9% saline daily for about a week (in three 30 minute intervals daily). Nebulizer devices for use in the aerosolization are available from Aerotech II, CIS-US, Bedford, Mass., with a nasal chamber adapted for murine nasal passages (e.g., a nose-only chamber from Intox Products, Albuquerque, N. Mex.). When driven by compressed air at a rate of 10 liters/min., the devices described produce aerosol particles having a median aerodynamic diameter of 1.4 .mu.m.

[0109] Control mice are preferably littermates which are protein-antigen challenged without prior immunization. For farther details concerning this animal model, those of skill in the art may wish to refer to Foster, et al. J. Exp. Med., 195-201, 1995; and, Corry, et al., J. Exp. Med., 109-117, 1996.

EXAMPLE II

Reduction of Eosinophil Accumulation in Lung Tissue in a Murine Asthma Model by Administration of ISS-ODN

[0110] BALB/c mice, 6-10 weeks of age, were prepared as models of allergic asthma as described in Example I (subcutaneous injection of OVA followed by antigen challenge at a concentration of 50 mg OVA/ml PBS). Prior to each inhalation with OVA according to this scheme, sets of 8 mice each were treated as described in the Table below. Control mice were antigen challenged but untreated and naive mice were not challenged with antigen. All ISS doses were 100 .mu.g per administration. Dexamethasone (a conventional steroidal anti-inflammatory used in the treatment of asthma) doses were 5 mg/kg/mouse. Priming doses of antigen were 25 .mu.g OVA adsorbed to alum in 0.2 ml phosphate buffered saline (PBS). Challenge doses of antigen were 10 ml of 50 mg OVA/ml PBS. IN=intranasal;

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IP=intraperitoneal; SC=subcutaneous and N/A=not applicable.

1 Set # Material Received Route and Timing 1 Naive mice (no antigen) N/A 2 DY1018 (ISS-ODN) IN, 1 day before the first inhalation 3 DY1018 IN, 1 day before the second inhalation 4 DY1018 IN, with the second inhalation 5 DY1018 IN, 2 days after the second inhalation 6 DY1018 IP, 1 day before the first inhalation 7 DY1018 IP, 1 day before the second inhalation 8 DY1018 IP, with the second inhalation 9 DY1018 IP, 2 days after the second inhalation 10 DY1018 IT, 2 days after the second inhalation 11 DY1019 (M-ISS-ODN) IN, 2 days after inhalation 12 DY1019 IP, 2 days after the second inhalation 13 DY1019 IT, 2 days after the second inhalation 14 Dexamethansone SC, 2 days after the second inhalation 15 Dexamethansone SC, 7 days after the second inhalation 16 control mice (antigen only) N/A

[0111] DY1018 has the nucleotide sequence:

[0112] 5'-TGACTGTGAACGTTCGAGATGA-3' (SEQ.ID.No. 19) with a phosphothioate backbone as the nucleotide sequence:

[0113] 5'-TGACTGTGAAGGTTGGAGATGA-3' (SEQ.ID.No. 20) with a phosphothioate backbone.

[0114] On day 32, each mouse was bled by tail snip (approximately 50 .mu.l volume) into a 0.1 mM solution of PBS and EDTA. Red blood cells in solution were lysed with 150 mM NH.sub.4Cl and 10 mM KHCO.sub.2O in dH.sub.2O then stained (Wright-Giesma stain). Lung lavage from each mouse was obtained after sacrifice by canalization of the trachea and lavage with 800 microliters PBS, then the lavage product was stained. Bone marrow samples from each mouse were obtained by flushing of extracted femur marrow with PBS. Histological specimens of lung and trachea tissue were obtained from the right lower lobe of the lung and trachea. Specimens were frozen, sectioned to a 5 micron width and stained with DAB peroxidase.

[0115] Results are expressed in the Table below as percent eosinophils compared to total leukocytes (inflammatory infiltrate) in each sample, except for the "lung" results, which represent the number of eosinophils per microscopic field (5 randomly selected fields were evaluated for each sample). In summary, the control mice had an average of 67% eosinophils in the lung/trachea tissue samples, while mice who received the mutant ISS-ODN (M-ISS-ODN; DY1019) had 52% and 88% (.+-.12%) average accumulation of eosinophils in lung tissue after IP and IN administration, respectively. The higher values for the mice treated with M-ISS-ODN after antigen challenge is most likely owing to the immunoinhibitory properties of DY1019 (see, the co-pending, commonly owned U.S. patent application entitled "Inhibitors of DNA Immunostimulatory Sequence Activity"; Eyal Raz, inventor; filed Jun. 6, 1997 (Serial No. 60/048,793)). Mouse sets 7 and 8 therefore model an partially immune incompetent host with allergic asthma.

[0116] In startling contrast, the mice pre-treated with the DY1018 ISS-ODN delivered intranasally had less than about 10% eosinophil accumulation in the lung and trachea when treated after antigen challenge and only about 19% eosinophil accumulation when treated before antigen challenge. These values represent up to an 80% reduction in eosinophil accumulation compared to the control mice and more than a 90% reduction in comparison to M-ISS-ODN (IN) treated mice.

[0117] The IP ISS-ODN treated mice fared even better, with a 6% eosinophil accumulation in the lung and trachea on treatment before and after antigen challenge. This value represents an 86% reduction in eosinophil accumulation as compared to the control mice and an 91% reduction as compared to M-ISS-ODN (IP) treated mice.

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[0118] These data indicate that the IL-5 stimulated eosinophil accumulation in lung tissue which characterizes the late phase of allergic asthma is inhibited by the ISS-ODN therapeutic methods of the invention.

2 Lung and Broncheoalveolar Tracheal Set # Bone Marrow Lavage Blood Tissue 1 (naive) 3 .+-. 2 0 2 .+-. 1 2 .+-. 1 2 (ISS) 5 .+-. 1 10 .+-. 2 3 .+-. 1 8 .+-. 1 3 (ISS) 12 .+-. 1 17 .+-. 4 6 .+-. 2 19 .+-. 5 4 (ISS) 5 .+-. 1 3 .+-. 1 2 .+-. 1 6 .+-. 1 5 (ISS) 8 .+-. 2 4 .+-. 3 3 .+-. 1 6 .+-. 4 6 (ISS) 10 .+-. 1 10 .+-. 1 4 .+-. 1 16 .+-. 4 7 (M-ISS) 13 .+-. 1 51 .+-. 3 10 .+-. 1 88 .+-. 12 8 (M-ISS) 13 .+-. 1 43 .+-. 3 10 .+-. 1 52 .+-. 14 9 (control) 3 .+-. 2 42 .+-. 4 14 .+-. 3 67 .+-. 5

EXAMPLE III

Antigen Independent Reduction of Eosinophil Accumulation in Lung Tissue

[0119] To evaluate whether the eosinophil suppression demonstrated by the data in Example II is dependent upon immune stimulation by the ISS-ODN, mice were sensitized to OVA using a conventional, Th2 stimulatory adjuvant (alum), treated with ISS-ODN or a control, and measured for eosinophil suppression before ISS-ODN stimulation of the mouse immune system would be expected to occur.

[0120] More specifically, groups of four mice were immunized with 25 .mu.g OVA in 1 mg alum by subcutaneous injection on days 1, 7, 14 and 21. This immunization protocol is known to stimulate a Th2 type response to the antigen in mice. On day 27, one group of animals received 100 .mu.g of the DY1018 ISS-ODN described in Example I by intraperitoneal administration. A control group received the mutant DY1019 M-ISS-ODN described in Example I by the same route.

[0121] On day 28, the animals in each group received 10 mg OVA/ml phosphate buffered saline by inhalation for 30 minutes. On day 30, some of the animals in each group received a second injection of ISS-ODN or M-ISS-ODN and the animals who had not been treated on day 27 were treated with ISS-ODN or M-ISS-ODN. The inhalation challenge with OVA was repeated on day 31 and the animals were sacrificed for eosinophil counting within 24 hours.

[0122] The results of this experiment are set forth in the Table below. Animals that received two treatments with ISS-ODN on days 27 and 30 had only 5.8%, eosinophils in the broncheo-alveolar fluid (BALF) lavage on day 32, even though immune stimulation by the ISS-ODN would be minimal so shortly after treatment. Even after only one treatment with ISS-ODN (on day 30), eosinophil accumulation in the BALF the treated animals was limited to 10.3%. In contrast, the control animals twice treated with M-ISS-ODN had 42.3% eosinophils in extracted BALF.

3 Treated on Animals Day 28 Blood Bone Marrow BALF ISS-ODN Yes 1.9% .+-. 0.8 5.8% .+-. 2.5 5.8% .+-. 2.8 M-ISS-ODN Yes 9.8% .+-. 2.1 13.0% .+-. 0.9 42.3% .+-. 3.5 ISS-ODN No 3.5% .+-. 0.6 10.5% .+-. 1.4 10.3% .+-. 1.3

[0123] These data establish that practice of the invention can inhibit allergic inflammation in animals and that the inhibition can occur as quickly as one day after treatment.

EXAMPLE IV

Selective Induction of a Th1 Response in a Host After Administration of an ISS-ODN Containing Plasmid

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[0124] In mice, IgG 2A antibodies are serological markers for a Th1 type immune response, whereas IgG 1 antibodies are indicative of a Th2 type immune response. Th2 responses include the allergy-associated IgE antibody class; soluble protein antigens tend to stimulate relatively strong Th2 responses. In contrast, Th1 responses are induced by antigen binding to macrophages and dendritic cells.

[0125] To determine which response, if any, would be produced by mice who received ISS-ODN according to the invention, nine groups of Balb/c mice were immunized with 10 .mu.g .beta.-galactosidase protein (conjugated to avidin; Sigma, St. Louis, Mo.) to produce a model allergic phenotype and treated as follows:

4 Mouse Group ISS-ODN Treatment 1 None (.beta.-gal) 2 DY1018 (ISS-ODN) injected with the antigen 3 DY1018 injected 72 hrs. after the antigen (same site) 4 DY1019 (M-ISS-ODN) injected with the antigen 5 DY1019 injected 72 hrs. after the antigen (same site)

[0126] At 2 week intervals, any IgG 2a and IgG 1 to (.beta.-galactosidase present in the serum of each mouse were measured by enzyme-linked immunoabsorbent assay (using antibodies specific for the IgG 1 and IgG 2A subclasses) on microtiter plates coated with the enzyme.

[0127] As shown in FIG. 2, only the mice who received the ISS-ODN produced high titers of IgG 2A antibodies, which increased in number over a period of 12 weeks. As shown in FIG. 3, immunization of the mice with the antigen itself or with the mutant ISS-ODN induced production of relatively high titers of IgG 1 antibodies. The data shown in the FIGURES comprise averages of the values obtained from each group of mice.

[0128] These data indicate that a selective Th1 response is induced by administration of an ISS-ODN according to the invention to an antigen-challenged host. Further, the data indicate that ISS-ODN administration according to the invention biases the immune system toward the Th1 phenotype on antigen challenge, even when the ISS-ODN are administered before antigen challenge (in this instance, 72 hours before challenge).

EXAMPLE V

Suppression of IgE Antibody Response to Antigen by Immunization with Antigen-Encoding Polynucleotides

[0129] To demonstrate the IgE suppression achieved through stimulation of a Th1 type cellular immune response in preference to a Th2 type cellular immune response, five to eight week old Balb/c mice were immunized with one of two recombinant expression vectors: ISS-ODN containing pCMV-Lac-Z (which contains two copies of nucleotide sequences similar to the DY1018 ISS-ODN) or a control plasmid, pCMV-BL. A third group of the mice received injections of antigen (.beta.-galactosidase). Plasmid DNA was purified and its endotoxin content reduced to 0.5-5 ng/1 mg DNA by extraction with TRITON X-114 (Sigma, St. Louis, Mich.). Before inoculation, pDNA was precipitated in ethanol, washed with 70% ethanol and dissolved in pyrogen free normal saline.

[0130] Immunization was by intradermal injection of plasmid DNA loaded onto separate tynes of a MONOVACC.RTM. multiple tyne device (Connaught Lab, Inc., Swiftwater, Pa.). Briefly, the tyne devices were prepared after extensive washing in DDW and overnight soaking in 0.5% SDS (sulfated dodecyl saline), washed again in DDW, soaked overnight in 0.1N NaOH, washed again in DDW and dried at 37.degree. C. for 8 hours. Six .mu.l of plasmid DNA dissolved in normal saline were pipetted onto the tynes of the tyne device just prior to each inoculation described below. The total amount of pDNA loaded on the device per inoculation was 25 .mu.g each of pCMV-Lac-Z and pCMV-BL. For

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purposes of estimating actual doses, it was assumed that less than 10% of the pDNA solution loaded onto the tyne device was actually introduced on injection of the tynes into intradermal tissue.

[0131] Each mouse was treated 3 times with 2 inoculations of each plasmid in a one week interval injected intradermally at the base of the tail. Another group of mice received a single intradermal injection in the base of the tail of 10 .mu.g of .beta. galactosidase protein (dissolved in 50 .mu.l of normal saline) in lieu of pDNA.

[0132] Toward inducing an IgE antibody response to subsequent sensitizing-antigen challenge, each group of mice was injected once intraperitoneally with 0.1 ml of phosphate buffered saline (PBS) solution containing 1 .mu.g of antigen (.beta. galactosidase; Calbiochem, San Diego, Calif.) and 3 mg of ALUM aluminum hydroxide as adjuvant (Pierce Chemical, Rockford, Ill.) 14 weeks after the initial immunization. Total IgE was assayed in sera from the mice 4 times over the subsequent 4 consecutive weeks.

[0133] IgE was detected using a solid phase radioimmunoassay (RAST) in a 96 well polyvinyl plate (a radioisotopic modification of the ELISA procedure described in Coligan, "Current Protocols In Immunology", Unit 7.12.4, Vol. 1, Wiley & Sons, 1994), except that purified polyclonal goat antibodies specific for mouse C chains were used in lieu of antibodies specific for human Fab. To detect anti-Lac-Z IgE, the plates were coated with .beta.-galactosidase (10 .mu.g/ml). The lowest IgE concentration measurable by the assay employed was 0.4 ng of IgE/ml.

[0134] Measuring specifically the anti-antigen response by each group of mice, as shown in FIG. 4, anti-Lac-Z IgE levels in the ISS-ODN containing plasmid injected mice were consistently low both before and after boosting (averaging about 250 CPM in RAST), while the protein injected mice developed high levels of anti-Lac-Z, particularly after the first antigen booster injection, when anti-Lac-Z levels in the mice rose to an average of about 3000 CPM. Consistent with acquisition of tolerance, anti-Lac-Z IgE levels in the protein injected mice declined over time, but continued to rise in the control mice who had not received any immunization to .beta.-galactosidase.

[0135] These data show that the ISS-ODN containing plasmid injected mice developed an antigen specific Th1 response to the plasmid expression product with concomitant suppression of IgE production, while tolerance was acquired in the protein injected mice only after development of substantially higher levels of antigen specific IgE antibodies.

EXAMPLE VI

IL-4, IL-5, IL-10 and INF.gamma. Levels, and CD4+ Lymphocyte Proliferation, in Mice After Delivery of ISS-ODN

[0136] BALB/c mice were injected intravenously with 100 .mu.g of DY1018, DY1019 or a random sequence control (DY1043) then sacrificed 24 hrs later. Splenocytes were harvested from each mouse.

[0137] 96 well microtiter plates were coated with anti-CD3 antibody (Pharmingen, La Jolla, Calif.) at a concentration of 1 .mu.g/ml of saline. The anti-CD3 antibody stimulates T cells by delivering a chemical signal which mimics the effects of binding to the T cell receptor (TCR) complex. The plates were washed and splenocytes added to each well (4.times.10.sup.5/well) in a medium of RPMI 1640 with 10% fetal calf serum. Supernatants were obtained at days 1, 2 and 3.

[0138] Th2 cytokine (IL-4, IL-5 and IL-10) levels were assayed in the supernatants using a commercial kit; Th1 cytokine (INF.gamma.) levels were assayed with an anti-INF.gamma. murine antibody assay

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(see, e.g., Coligan, "Current Protocols in Immunology", Unit 6.9.5., Vol. 1, Wiley & Sons, 1994). Relatively high levels of IL-4 and IL-10 with low levels of INF-.gamma. would be expected in mice with a Th2 phenotype, while relatively low levels of IL-4 and IL-10 with high levels of INF-.gamma. would be expected in mice with a Th1 phenotype. Relatively high levels of IL-5 characterize a proinflammatory milieu, while the converse is true of relatively low levels of IL-5.

[0139] As shown in FIGS. 5 and 6, levels of anti-CD3 stimulated IL-4 and IL-10 secretion in DY1018 treated mice were substantially lower than in the control mice. Levels in the DY1019 mice were intermediate. Levels of pro-inflammatory IL-5 were reduced in DY1018 treated mice to a comparable extent (FIG. 7).

[0140] Levels of T cell proliferation in response to antigen challenge were greatly reduced in DY1018 (ISS-ODN) treated mice as compared to DY1019 (mutant ISS-ODN) treated and control mice. This suppression of T cell proliferation was reversible on administration of IL-2, demonstrating that the suppression was due to Th2 anergy in the ISS-ODN treated mice (see, Table below).

5 Treatment Control (CPM) ISS-ODN (CPM) M-ODN (CPM) OVA (50 .mu.g/ml) 40680 .+-. 5495 15901 .+-. 4324 42187 .+-. 13012 OVA + IL-2 (1.5 65654 .+-. 17681 42687 .+-. 6329 79546 .+-. 10016 ng/ml) OVA-IL-2 (15 60805 .+-. 19181 57002 .+-. 10658 60293 .+-. 5442 ng/ml)

[0141] Levels of Th1 stimulated IFN-.gamma. secretion were greatly increased in the DY1018 treated mice, but substantially reduced in the DY1019 treated mice (as compared to the control), indicating stimulation of a Th2-type milieu in the latter mice (FIG. 8). Additional data demonstrating these results are shown in the Table below. "b/f" in the Table refers to before; "1st", "2nd" and "each" refer to administration of the compound before the 1st or 2nd antigen challenge.

[0142] Importantly, treatment of mice before antigen challenge is even more effective in shifting the immune response on antigen challenge to a Th1 phenotype than is post-challenge treatment. As shown in FIGS. 9 and 10, antigen primed (but unchallenged) animals injected with ISS-ODN DY1019 72 hours before antigen challenge (with .beta. galactosidase) mounted a more robust Th1-type immune response to the antigen than did their post-challenge treated littermates or littermates treated pre-challenge with a mutant, inactive oligonucleotide (DY1019), as measured by increased IFN.gamma. secretion (FIG. 9) and CD4+ lymphocyte proliferation (FIG. 10).

6 Set # IL-5(pg/ml) IFN-.gamma.(pg/ml) 1 (naive) <20 <20 2 (ISS) in b/f 1st 466 .+-. 40 246 .+-. 86 3 (ISS) in b/f 2nd 531 .+-. 109 168 .+-. 22 4 (ISS) in with 2nd 575 .+-. 90 98 .+-. 44 5 (ISS) in b/f each 200 .+-. 66 443 .+-. 128 6 (ISS) ip; b/f 1st 190 .+-. 52 664 .+-. 61 7 (ISS) ip; b/f 2nd 421 .+-. 102 252 .+-. 24 8 (ISS) ip; with 2nd 629 .+-. 110 104 .+-. 15 9 (ISS) ip; b/f each 121 .+-. 18 730 .+-. 99 10 (ISS) it; b/f each 191 .+-. 49 610 .+-. 108 11 (M-ISS) in; b/f each 795 .+-. 138 31 .+-. 22 12 (M-ISS) ip; b/f each 820 .+-. 122 33 .+-. 33 13 (M-ISS) it; b/f each 657 .+-. 52 102 .+-. 57 14 (steroid) sc; b/f each 424 .+-. 90 <20 15 (steroid) sc; daily 252 .+-. 96 <20 16 (control) not treated 750 .+-. 124 24 .+-. 21

[0143] Further, ISS-ODN administered according to the invention suppress Th2 cytokine release from Th2 sensitized mouse cells (splenocytes harvested from OVA-primed mice, then incubated for 72 hours with 100 .mu.g/ml OVA in vitro). ISS-ODN treatment took place either 1 (-1) or 3 (-3) days before sacrifice. These data are shown below:

7 Group IL-3 (pg/ml) IL-5 (pg/ml) IFN-.gamma. (pg/ml) Control 1299 .+-. 89 657 .+-. 52 <20 ISS-ODN (-1) 309 .+-. 26 112 .+-. 18 <20 ISS-ODN (-3) 463 .+-. 48 144 .+-. 27 <20 ISS-ODN (-1) 964 .+-. 81 508 .+-. 77 <20

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